# HDX-MS Analytical Instrument for Incorporation on a Robotic Rail, Featuring Dual Enzyme Chambers and HPLC Separations at -30 °C

# Designed by Dr. Jeffrey W. Hudgens

#### Construction instructions are within three documents:

1.	The HPLC box and cooling system (this document)?	217	pages
2.	The Multi-channel Temperature Controller	31	pages
2	The Multi-channel Valve Controller	26 1	2000

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#### **Design Objectives & Integration Plan**

- Maintain height & depth of current cold box & provide mounting brackets to robot rail.
- Minimize back-exchange by use optimum temp zone scheme.
- Operate electrochemical cell at low T.
- Provide a permanent "store-in-place" facility for idle enzyme columns (providing constant H<sub>2</sub>O flow and low T).
- Allow data collection with one or two enzyme columns.
- Improve HPLC resolution (but don't sacrifice speed).
- Provide electrospray solvent rebalancing and supercharging capability by adding solvent mixes to analyte before entering ESI source.
- Test for robust hardware & software operation before installing into the current HDX-MS instrument.

These were the initial objectives. Minimization of carry-over is also an implicit objective.

#### **Comments on the Design and Construction**

- The NIST housings were printed on TAZ 5 and TAZ 6 3D-printers in PLA
  plastic. All were serviceable but other plastics may work better. Large
  objects in PLA will not conform to dimensions exactly, thus, I have adjusted
  some parts so that they fit. PLA has some elasticity which will allow you to
  force the housing to fit the other parts.
- Most STL files fit on the TAZ platen. For the mask used on the door I have included a larger STL file that will allow production on a larger format printer.
- For some parts I have included both STL and IGS files. For the prototype these were fabricated in the machine shop; however, these may be more cheaply produced via 3D printing in steel or aluminum.
- Keep the coolant hoses as short as is practical. Hoses account for much of the loss of cooling capacity.
- Use foam sealing, where possible, particularly, on the surfaces of the PLA housing.
- When cooling the system, purge the box with dry gas, such as the boiled-off liquid N<sub>2</sub>.
- The protease assembly allows precise control of the protease temperature.
   However, each vendor offers a protease column manifesting a unique
   profile (diameter and length). To standardize the profile presented to the
   protease clamp assembly, a two-piece silver or aluminum collet envelopes
   the protease column. The collet presents as a 0.65" OD cylinder. Thus, each
   protease column will require its own collet. Three collet designs are offered
   in the STL files.
- During operation the idle column has a flow of cleaning solution passing through it.

**TABLE 1: Cross-reference Table between Drawings and STL File Name** 

No.	Drawing Name	STL File Name
1	-30C Cover	(-30C)_Cover.STL
2	Accessory box (part 1)	Accessory box (part 1).STL
3	Accessory box (part 2)	Accessory box (part 2).STL
4	Accessory box (part 3)	Accessory box (part 3).STL
5	Accessory box (part 4)	Accessory box (part 4).STL
6	Accessory Mixer Sole Plate	Accessory Mixer Sole Plate.STL
7	Adapter to Accessary Box	Adapter to Accessary box adapter.STL
8	Aux Heater adapter	Aux Heater adapter.STL
9	Backside housing (left-4)	Backside housing (left-4).STL
10	Backside housing (right)	Backside housing (right).STL
11	Backside housing 2	Backside housing metal (left-2).STL
12	Backside housing Left (Alt-design)	Backside housing Left-3a- version_3).STL
13	Backside housing metal2 (left-1)	Backside housing metal2 (left-1).STL
14	Backside metal	Backside housing (left-3a).STL
15	Cold Chamber exit tube	Cold Chamber exit tube.STL
16	cooling tube clamp	cooling tube clamp.STL
17	cover right metal	cover right metal.STL
18	cover(-30C) base	cover(-30C).STL
19	cryo-endcap part 1	cryo-endcap part 1.STL
20	cryo-endcap part 2	cryo-endcap part 2.STL
21	d-sub 9-pin Connector Mount	d-SUB MOUNT(0C).STL
22	Exit port washer	Exit port washer.STL
23	EyGly mixer clamp	mixer clamp.STL
24	EyGly Mixer holder	Mixer holder.STL
25	fan tunnel top(-30)	fan tunnel top(-30).STL
26	HPLC Column clamp (part 1)	HPLC Colum clamp (part 1).STL
27	HPLC MOUNT clamp	HPLC MOUNT clamp.STL
28	internal (-30C) cap	internal (-30C) cap.STL
29	mask_front3(LEFT)	mask_front3(LEFT).STL
30	mask_front3(RIGHT)	mask_front3(RIGHT).STL
31	Power supply standoff	Power supply standoff.STL
32	Shell-back metal	Shell-back metal.STL
33	Side Cover	Side Cover.STL
34	union holder optimize (bottom)	union holder optimize (base).STL
35	union holder optimize (TOP)	union holder optimize (TOP).STL

36	Valve adjust sleeve	Valve adjust sleeve.STL
37	valve wrench	wrench.STL
38	ACC Mixer Clamp	ACC Mixer Clamp.stl
39	Analytical Chamber Mask	mask2(0C).STL
40	Backside housing top metal	Backside housing top metal (left back).STL
41	coolant exit plate	Cryobulkhead.STL
42	Cryo-Bulkhead	Cryo-Bulkhead metal.STL
43	Door Mask Cover	mask3_cap.STL
44	Dust Protector for Injection Port	Funnel.STL
45	Electrical Cover	Elect Cover.STL
46	Left Mask (0C)	mask4a(0C).STL
47	Lock-ring of Dust Protector for Injection Port	Funnel_lock_ring.STL
48	mask_front3	mask_front3.STL
49	protease bearing well	Protease bearing well.STL (preferred)
50	Reducing cell holder	Antec bottom holder.STL
51	RTD Platform	RX402_mount.STL
52	Snout (Removable)	Accessory box (part 1b).STL
53	Valve Actuator	thotler valve driver.STL
54	Valve Plate #2 Fan tunnel (bottom)	fan tunnel (bottom).STL
55	Valve Plate #3 Fan tunnel (bottom)	fan tunnel bottom(-30).STL
56	Waste Hose Clamp	Acc Hose Clamp.STL

**TABLE 2: Cross-reference Table between Drawings and IGS File Name** 

No.	Drawing Name	IGS File Name
1	Accessary Box Adapter	Accessary box adapter.IGS
2	Bracket (Plate #2)	Bracket (Plate #2).IGS
3	Capillary Clamp Riser	capillary clamp riser(-30C).IGS
4	Central rail (bottom)	Central rail (bottom)_2.IGS
5	Central rail (top)	Central rail (top)_2.IGS
6	cooling tube clamp	cooling tube clamp.IGS
7	cryoside strut	Cryo-side.IGS
8	cyrotop strut	cryotop.IGS
9	Enzyme Reactor Standoff II	enzyme stand-off.IGS
10	Enzyme Sole Plate	Enzyme Plate.IGS
11	Extension Top Plate	motor box extention top.IGS
12	Fan Mount (Plate #2)	fan mount (0C).IGS
13	Fan riser (part 1)	fan riser(-30C)-fan mount.IGS
14	Fan riser (part 2)	fan riser(-30C)-part2_fan mount.IGS
15	Fan riser (part 3)	fan riser(-30C).IGS
16	Guard Trap Mount	Mount Trap Column.IGS
17	HPLC Colum clamp (part 1)	HPLC Colum clamp (part 1).IGS
18	HPLC Colum clamp (part 2)	HPLC Colum clamp (part 2).IGS
19	left lock plate(-30C)	left lock plate(-30C).IGS
20	Mixer Clamp	Mount Trap Column top.IGS
21	Motor Assembly to Rail Adapter	motor assembly to rail adapter.IGS
22	motor box extension	motor box extention.IGS
23	motor box side panel (interior)	motor box side panel (interior).IGS
24	Motor box side panel (outside)	motor box side panel.IGS
25	Motor Rail Base	motor rail base.IGS
26	Protease Base Plate	Enzyme Base Plate.IGS
27	Protease Bearing Well	protease bearing well.IGS (Use STL)
28	Protease Column BottomClamp	Column bottom1 Clamp3.IGS
29	Right Lock Plate	right lock plate.IGS
30	right lock plate(-30C)	right lock plate(-30C).IGS
31	Standoff valve plate #3	Plate #3 standoff.IGS
32	Top Clamp Assembly for Protease Column	Column top1 Clamp3.IGS
33	Valve #3 Plate	Plate(-30C).IGS
34	Valve Plate #2	three in Ine.IGS

#### NIST -30 °C HPLC for HDX-MS

-- Optimal system for mAb analyses

- Operation at -30 °C reduces H/D back-exchange rates by x40 as compared to 0 °C operation.
- Greater peptide LC peak capacity is obtained when using 40 min LC gradients with ≈ 5% back-exchange.
- Dual enzyme columns allow complex protein/glycan digestion schemes.
- LC carryover is minimized by cleaning the protease and analytical columns with 4 quaternary LC pumps.
- Publically-available schematics allow fabrication of this system through 3D printing and robotic machining.

Robot Arm
Input Port

Two enzyme column nooks

0 °C nook for fluidic parts & reducing cell

-30 °C chamber contains 1500 bar analytical column

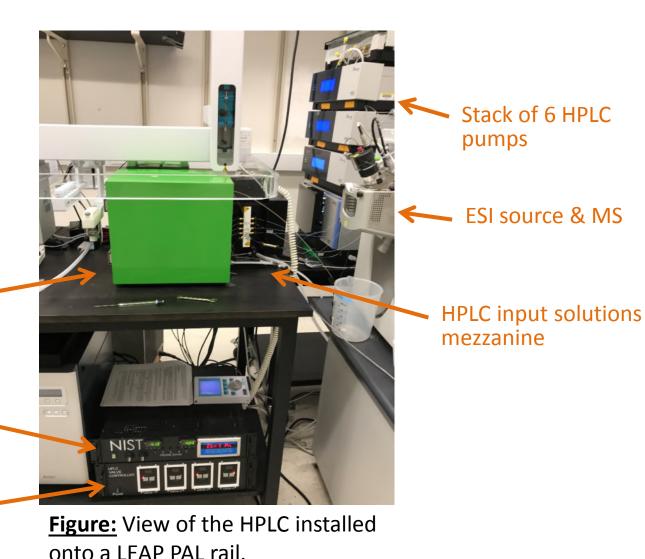
HPLC mounted on robotic rail of the HDX-MS system.

7-zone Temp.
Controller
(< ±0.1°C)



MATERIAL MEASUREMENT LABORATORY

## NIST Cold (-30 °C) HPLC for HDX-MS



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Cold HPLC

9-zone

Valve

Controller

Temperature Controller

### Features of the NIST Cold HPLC

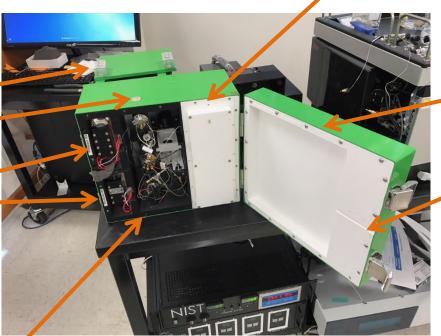
Guard & analytical column chamber

Adapter rail clamps \_\_\_\_

Injector input port

Protease column stages

Chamber for 0 °C fluidic components & reducing cell

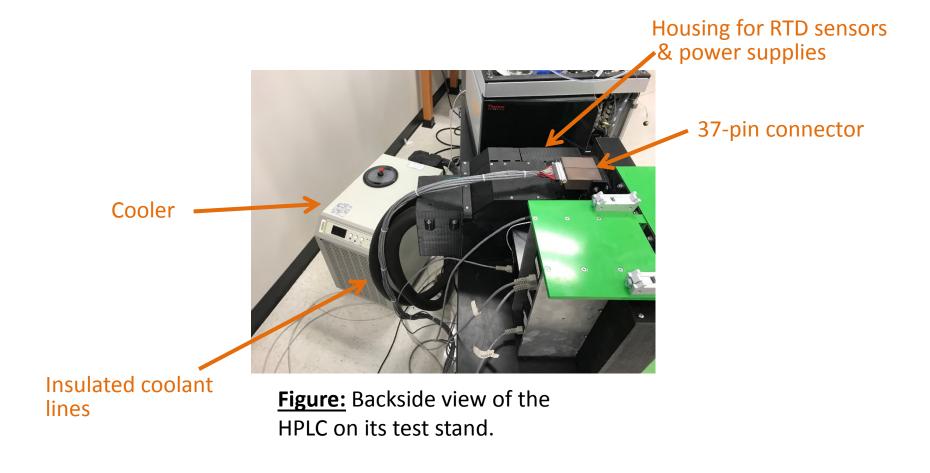


**Figure:** View of the opened HPLC on its test stand.

Insulated lift-off door

Groove for cable to electrolytic reducing cell

## Backside view of NIST HPLC



#### Rear View of NIST HPLC

37-pin cable seated into a 3-D printed steel mount

Adapter rail clamps

Housing for RTD sensors & power supplies

Insulated chamber housing coolant throttle valve & manifold

Chamber housing for detachable connectors of the coolant circuits

<u>Figure:</u> Backside view of the HPLC on its test stand.

Rail clamps

Mounts for valve motors #1, #4, #2

Mounts for valve motor #3

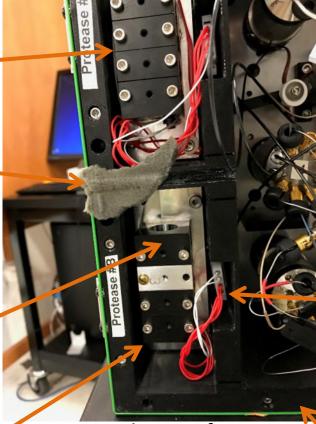
#### Detailed View of Protease Stages

For temperature stability 4 clamps hold the protease column collet assembly

Groove passes cable For reducing cell.

For temperature stability each protease column is placed into a collet adapter & the assembly is clamped to the stage.

Each protease stage pulls out on linear bearings

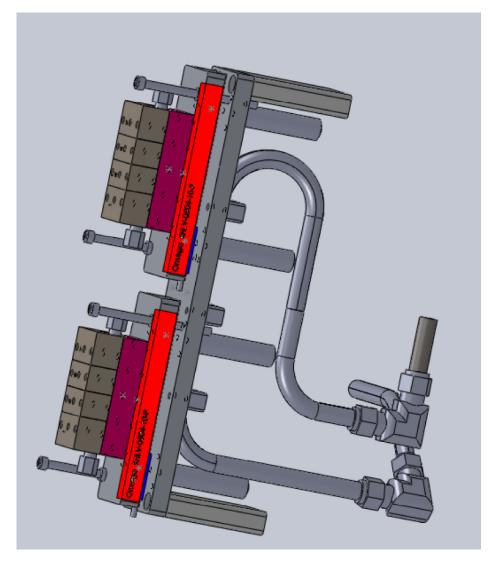


**<u>Figure:</u>** Detail view of protease chambers. Temperature of each protease stage is independently selected and controlled.

4-contact plastic connector for heater and sensor circuits. (Female connector is panelmounted to 3-D printed mask

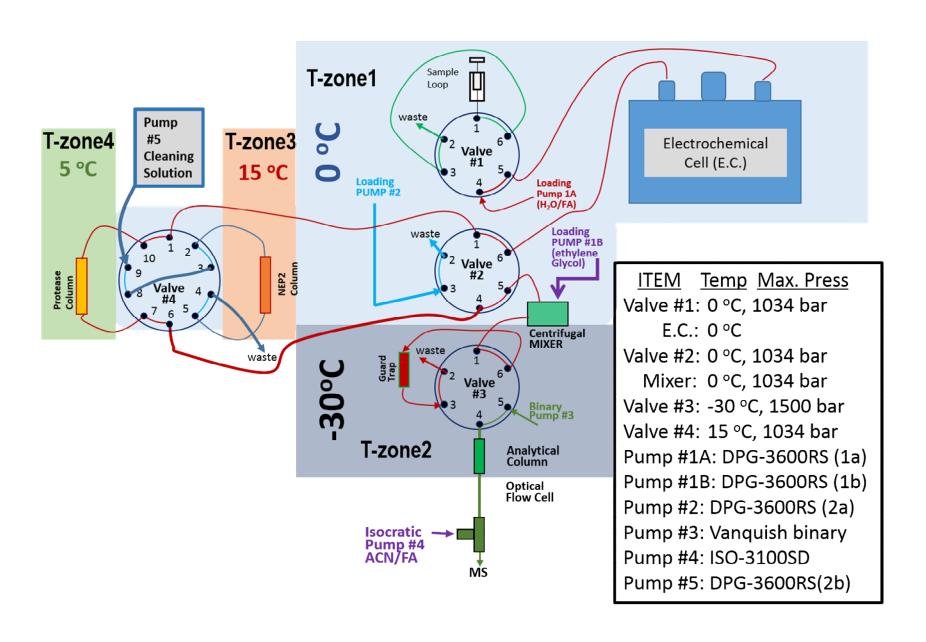
3-D printed (PLA) mask

# View of Protease columns on extendable mounts with Kapton heating Strips



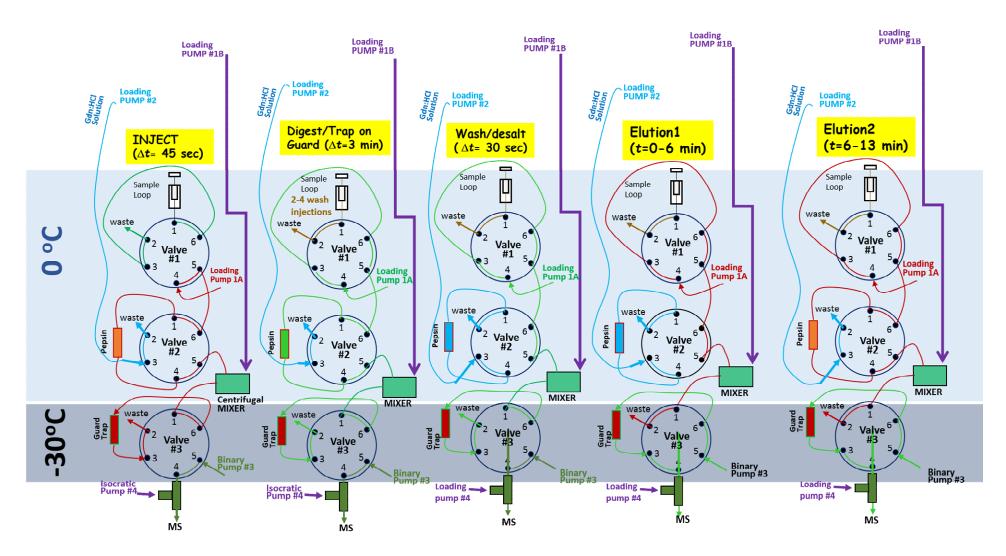
View of Protease columns on extendable mounts with Kapton heating Strips. (Thermister and additional heaters are on underside of each cassette.

#### Overview of the HPLC Fluidic System



#### Valve States During Proteomic Analyses

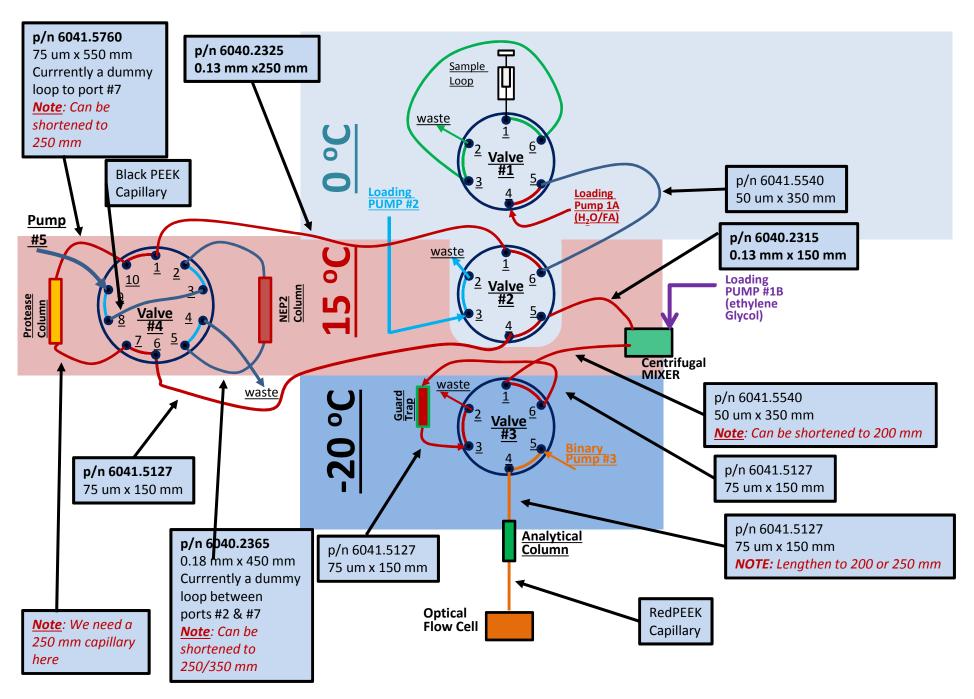
-- Valve 4 is not shown, but in this diagram it would be in the position of the pepsin column.



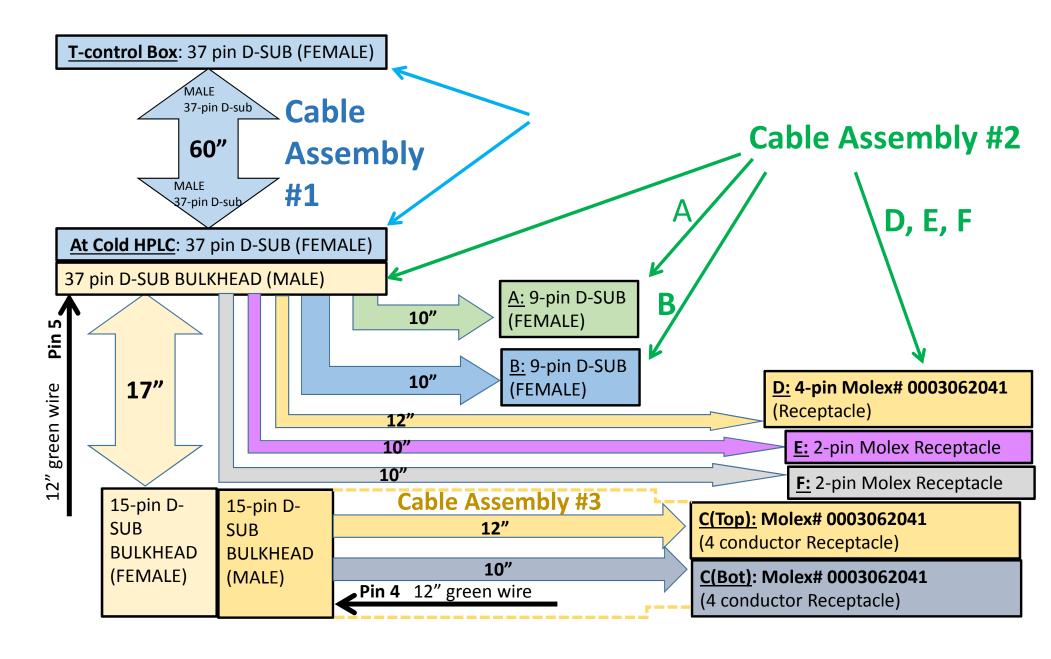
#### Analytic Fluidic Circuits of the HPLC

Connection #	Circuit	Function	Valve	Port	Pressure	Off Valve Location	Length (mm)	Connector	
1	1	Injection to sample loop	1	1	nil		0	External port injection shaft	
2	1	Sample loop (in)	1	6	10K		50 uL loop	Regular	
3	1	Sample loop out)	1	3	10k		50 uL loop	Regular	
4	1	waste	1	2	10K	Acc. Box Waste Port	713.994	Regular (one end only)	
5	2	Loading Pump 1A (H2O/FA) source	1	4	10K	Acc. Box Union #5	713.994	nanoViper to nanoViper	
6	2	Source to Electrochem Cell	1	5	10K	Electrochem Cell or V2, p6		nanoViper to nanoViper	
7	2	Electrochem Cell to V2	2	6	10K	Electrochem Cell or V2, p6		nanoViper to nanoViper	
8	2	source to Valve #4	2	1	10K	Valve #4, p1		nanoViper to nanoViper	
9	2	Loading Pump #2A (H2O/FA) source	2	3	10K	Acc. Box Union #2	611.886	nanoViper to nanoViper	
10	2	Waste	2	2	nil	Acc. Box Waste Port	611.886	Regular (one end only)	
11	3	Valve #4 (out)	2	4	10K			nanoViper to nanoViper	
12	3	Valve #2 (out)	2	5	10K	To mixer Tee		nanoViper to nanoViper	
13	4	Loading Pump 1B (EG/MeOH/FA) source				Acc. Box Union #3 to Mixer Tee	625.856	nanoViper to nanoViper	
14	4	Mixer (out) to valve #3	3	1	10K	Mixer		nanoViper to nanoViper	
15	4	source to guard trap	3	6	22K	guard trap		nanoViper to nanoViper	
16	4	guard trap (out) to V#4	3	3	22K	guard trap		nanoViper to nanoViper	
17	4	waste	3	2	10K	Acc. Box Waste Port		Regular (one end only)	
18	5	Binary Pump #3 (EG/MeOH/H2O/FA) source	3	5	22K	Acc. Box Union #1	556.006	nanoViper to nanoViper	
19	5	To Analytical column	3	4	22K	Analytical column		nanoViper to nanoViper	
20	5	Analytical column			10K	Acc box mixer Tee		nanoViper to nanoViper	
21	6	Isocratic Pump #4 (H2O/MeOH/FA) source			10K	Acc. Box Union #6		nanoViper to nanoViper	
22	6	Acc box mixer Tee			10K	Mass Spectrometer		nanoViper to nanoViper	
23	7	Sample In from V2, P1	4	1	10K	V2, P1		nanoViper to nanoViper	
24	7	out to Proteolysis Column #1	4	10	10K	Proteolysis Column #1		nanoViper to nanoViper or Regular/Waters	
25	7	in from Proteolysis Column #1	4	7	10K	Proteolysis Column #1		nanoViper to nanoViper or Regular/Waters	
26	7	Prot Column (Out)	4	6	10K	to V2, P4			
27	8	Loading Pump #2B (H2O/FA) source	4	9	10K	Acc. Box Union #4	662.432	nanoViper to nanoViper	
28	9	Bridge (out)	4	8	10K	V4,P3		nanoViper to nanoViper	
29	9	Bridge (in)	4	3	10K	V4,P8			
30	9	Out to Protease Column #2	4	2	10K	Protease Column #2 (in)		nanoViper to nanoViper or Regular/Waters	
31	9	From Protease Column #2	4	5	10K	Protease Column #2 (out)		nanoViper to nanoViper or Regular/Waters	
32	9	Waste	4	4	10K	Acc. Box Waste Port	662.432	Regular (one end only)	
Connection #		Function	Valve #	Port	Pressure	Off Valve Location	Length (mm)		Connector
SOURCE LINES		Discos D. 112 (50/h4 011/1120/54)	•	_	221/	Ass. Dec. Halles #4	FFC		
18	5	Binary Pump #3 (EG/MeOH/H2O/FA) source	3	5	22K	Acc. Box Union #1	556 613		nanoViper to nanoVipe
9	2	Loading Pump #2A (H2O/FA) source	2	3	10K	Acc. Box Union #2	612		nanoViper to nanoVipe
13	4	Loading Pump 1B (EG/MeOH/FA) source			401/	Acc. Box Union #3 to Mixer Tee	626		nanoViper to nanoVipe
27	8	Loading Pump #2B (H2O/FA) source	4	9	10K	Acc. Box Union #4	662		nanoViper to nanoViper
5	2	Loading Pump 1A (H2O/FA) source	1	4	10K	Acc. Box Union #5	714		nanoViper to nanoVipe
WASTE								L (in)	
4	1	waste	1	2	10K	Acc. Box Waste Port	714		Regular (one end only)
10	2	Waste	2	2	nil	Acc. Box Waste Port	612	24.1	Regular (one end only)
17	4	waste	3	2	10K	Acc. Box Waste Port	556		Regular (one end only)
32	9	Waste	4	4	10K	Acc. Box Waste Port	662		Regular (one end only)

#### Capillary Circuit & Part Numbers



#### Diagram of the electrical Cable Assignments (Unverified)



#### Heater & Sensor Wiring Table

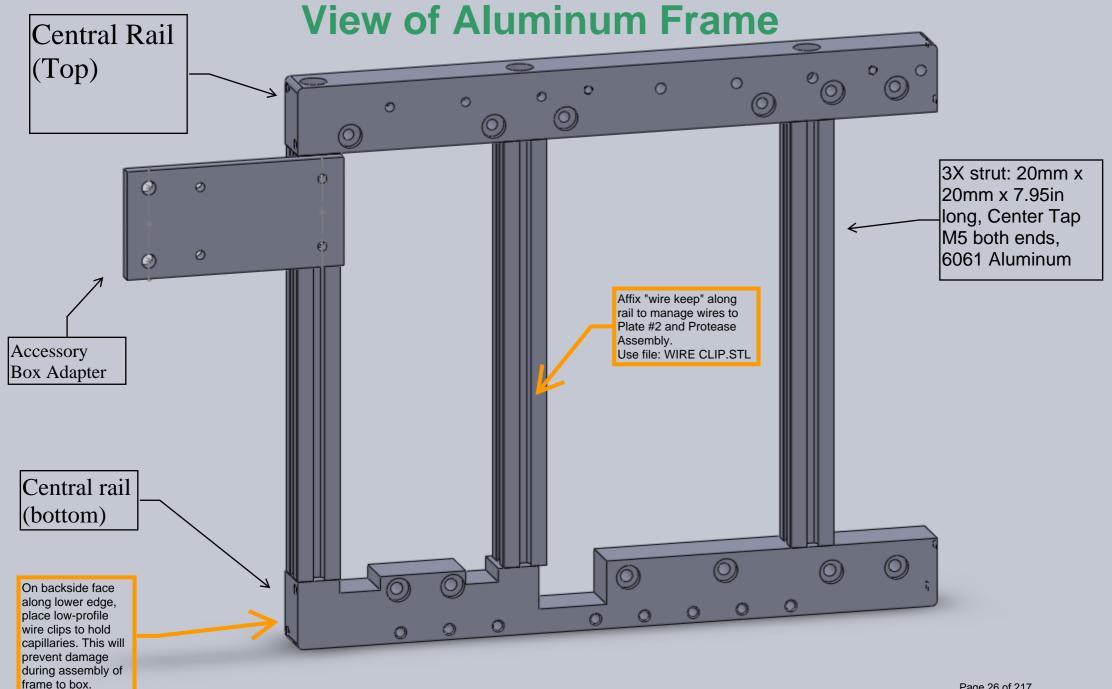
ZONE FUNCTION 37-PIN LENGT TERMINATINGFEMAL MALECONNECTO					TERMINATINGTyp	
	FONCTION	CONNECTO R	H & WIRE GAUGE (IN)	E CONNECTOR (PINS)	MALECONNECTO R (PINS)	e e
A				9-pin SubD Female (A)	9-pin SubD Male	
(-30°C)	The amoral at a m1/1			1	(A)	
	Thermistor1(+			6 2, 7	1 6	
	Thermistor1(-)			3, 8	2, 7	
	Heater1(+)			5	3, 8	
	Heater1(-)			9	5	
	Fan1(+)			8	9	
	Fan1(-)				8	
В	Ground			9-pin SubD Female (B)	9-pin SubD Male	
D				<u> 3-ріп заво Репіаїе (в)</u>	(B)	
	Thermistor2(+			6	1	
	)			2, 7	6	
	Thermistor2(-)			3, 8	2, 7	
	Heater2(+)			5	3, 8	
	Heater2(-) Fan2(+)			9 8	5   9	
	Fan2(-)			8	8	
	Ground				_	
C(top				15-pin DIN Female (C)	15-pin DIN Male	Plastic Male
)				(Amphenol FCI Part#	(C)	Connector (D)
				G17S1500110EU) 1	(Amphenol FCI Part#	(Molex Part# 0050291758)
				9	DA15P064TXLF)	1
	Thermistor3(+			2, 10	1	2
	)			3, 11	9	3
	Thermistor3(-)			4	2, 10	4
	Heater3(+)				3, 11 4	
	Heater3(-) Ground				4	
C(Bot)	Ground			15-pin DIN Female (C)	15-pin DIN Male	
	Thermistor4(+			8	(C)	
	)			15	8	
	Thermistor4(-)			6, 13	15	
	Heater4(+)			7, 14	6, 13	
D	Heater4(-)			Plastic Female	7, 14 Plastic Male	
				Connector(D)	Connector (D)	
				(Molex Part#	(Molex Part#	
				0003062041)	0050291758)	
	Thermistor5(+			1	1	
	Thormistors()			2	2	
	Thermistor5(-) Heater5(+)			3 4	3 4	
	Heater5(-)			7	7	
	Ground					

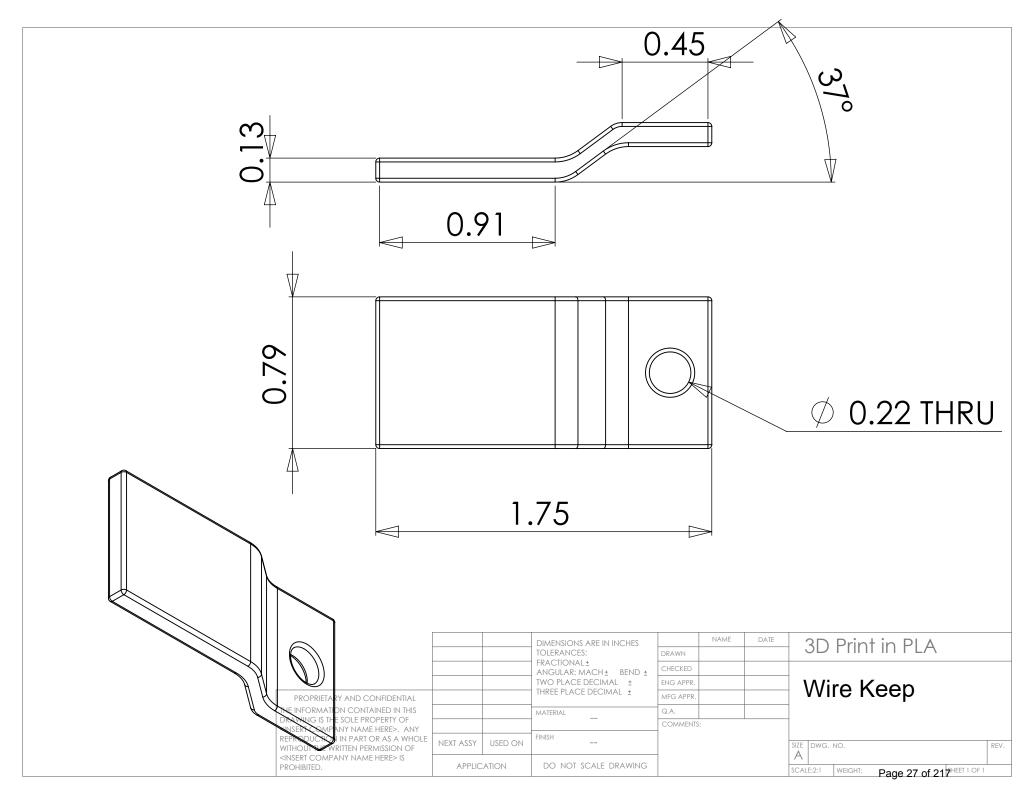
#### Commercial Parts used to Construct the NIST Cold HPLC (page 1/2)

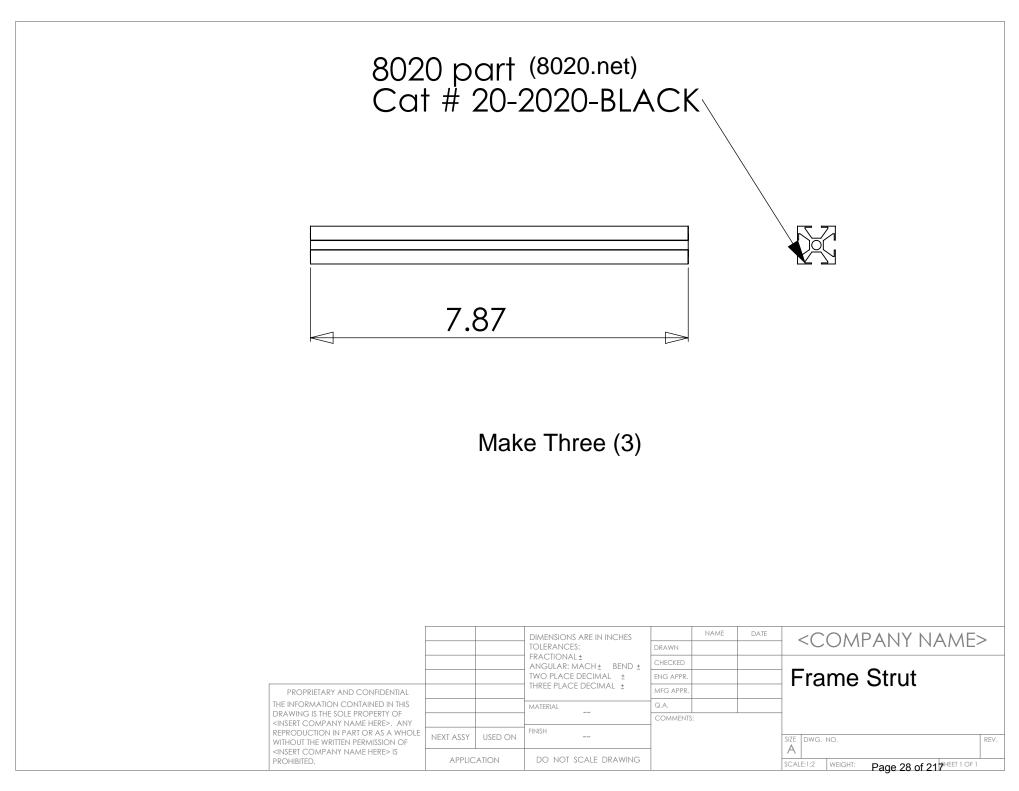
Item #	Part #	Description	Vendor	QNTY	NOTES
		NOTE: Thermal sensors & heaters are listed on the T-controller parts list.			
1	Trajon_holder	Collet for Trajon Protease column	Shapeways	2	Item can be printed by any vendor that can print aluminum
2	Mixer Holder	Holder for EtGly/H2O solution mixer	Shapeways	1	Item can be printed by any vendor that can print aluminum
3	Mixer Clamp	Clamp for mixer holder	Shapeways	1	Item can be printed by any vendor that can print aluminum
4		12" x 48" x 10 mm Spaceloft® Blanket	http://buyaerogel.com/	1	Calculate what you need. See notes on this issue in the prints. See Table of Contents
5		12" x 48" x 5 mm Spaceloft® Blanket	http://buyaerogel.com/	1	Calculate what you need. See notes on this issue in the prints. See Table of Contents
6	Model# RS44CL0	RS Recirculating Chiller, provides about 285W @ -30 $^{\circ}\text{C}$	SP Scientific, FTS Systems Thermal Products	1	Requires 208V, single phase.
7	RNE-0844	Arctic Tubing, 3/8" I.D, 1 foot by Good Year	SP Scientific, FTS Systems Thermal Products	8	Not listed:
8	RUR-0803	Insulation Tube, 1 foot length, 3/4" I.D. x 3/8" Wall for Use with RNE-0844 Tubing	SP Scientific, FTS Systems Thermal Products	8	<ol> <li>Hinge model</li> <li>Swagelock adapter to Shell</li> </ol>
9	60794	1/2" NPT HFC 35 Series Polysulfone Coupling Insert - Shutoff	United States Plastic Corp, www.usplastic.com	1	3. D-sub connectors
10	60670	3/8" In-Line Hose Barb HFC 35 Series Polysulfone Coupling Body - Shutoff		1	4. 9-pin connector on side.
11	60667	1/2" NPT HFC 35 Series Polysulfone Male Coupling Body - Shutoff	United States Plastic Corp, www.usplastic.com	1	5. Wire types.
12	60797	3/8" In-Line Hose Barb HFC 35 Series Polysulfone Coupling Insert - Shutoff	United States Plastic Corp, www.usplastic.com	1	6. Swage SS Tube Fitting, Female Connector, 3/8 in. Tube OD x 1/2 in. Female NP
13	SS-600-7-8	3/8" Swagelock x 1/2" Female NPT Straight	Dibert Valve & Fitting Co., Inc.,	2	Used to interface with Polysulfone couplers
			http://dibert.swagelok.com		
14	SS-810-3-6-6	Reducing Union Tee, 1/2 in. x 3/8 in. x 3/8 in. Tube OD, Swagelok Fittings	Dibert Valve & Fitting	2	Used in counterflow heat recapture assembly
15	SS-600-9	Union Elbow, 3/8 in. Tube OD, Swagelok Fittings	Dibert Valve & Fitting	12	
16	SS-T6-S-035-20	316/316L Stainless Steel Seamless Tubing, 3/8 in. OD x 0.035 in. Wall x 20 Feet	Dibert Valve & Fitting	1	You will need to get quote for this item.
17	SS-43GS6-LL	1-Piece 40G Series 2-Way Ball Valve, 1.5 Cv, 3/8 in. Swagelok Tube Fitting, Black Latch Lock Handle	Dibert Valve & Fitting	1	I have two in my system, and I believe only one is needed.
18	SS-600-SET	316 Stainless Steel Ferrule Set (1 Front Ferrule/1 Back Ferrule) for 3/8 in. Swagelok Tube Fitting	Dibert Valve & Fitting	10	If you are perfect, you won't need these.  One mistake, you will likely need it. Order in multiples of 10.
19	93365A130	6.32 x 0.150" heat-set insert	McMaster-Carr	2	
20	93365A132	6-32 x 0.250" Heast-set Insert for Plastic	McMaster-Carr	2	
21	93365A122	4-40 Heat-set Insert for Plastics	McMaster-Carr	1	
22	93365A140	8-32 X 0.185" Heat-set Insert for Plastic	McMaster-Carr	2	
23	91253A145	6-32 x 5/16" Alloy Steel Hex Drive Flat Head Screw	McMaster-Carr	1	These work, but I don't like them. I recommend a Phillips head.
24	91253A146	Black-Oxide Alloy Steel Hex Drive Flat Head Screws	McMaster-Carr	1	These work, but I don't like them. I recommend a Phillips head.
25	20-2020-BLACK-FB,	pa Strut, 20mm x 20mm x 7.95in long, Tap M5 both ends, 6061 Alun	8020, Inc	3	<u>www.8020.net</u> 3

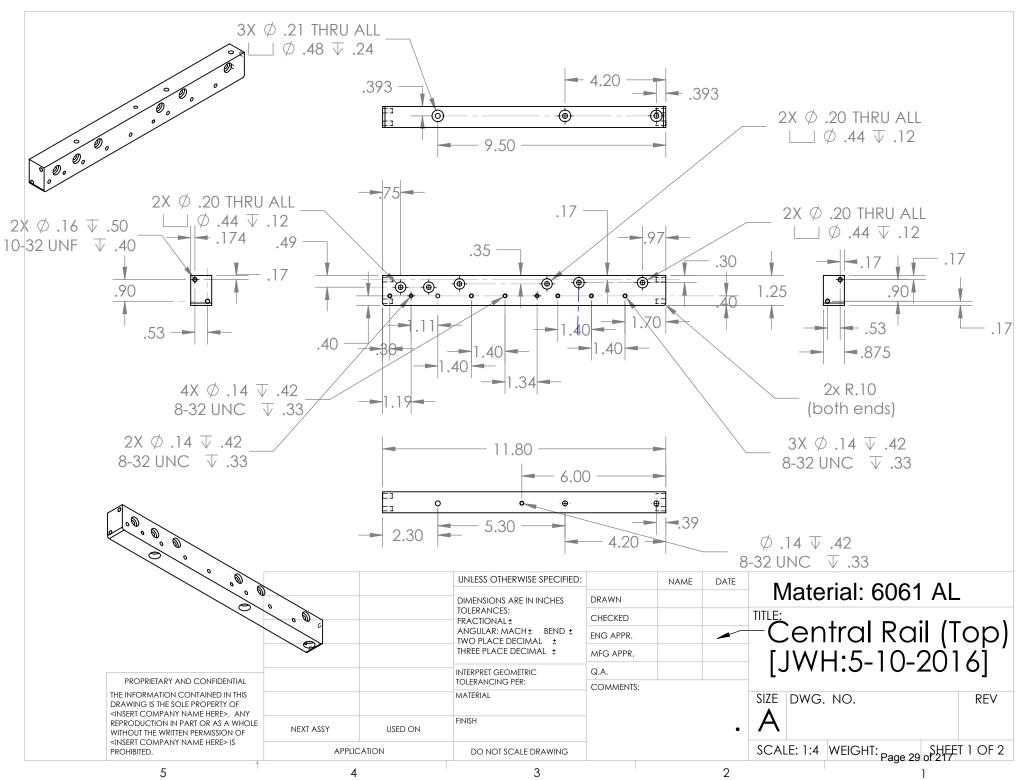
#### Commercial Parts used to Construct NIST Cold HPLC (page 2/2)

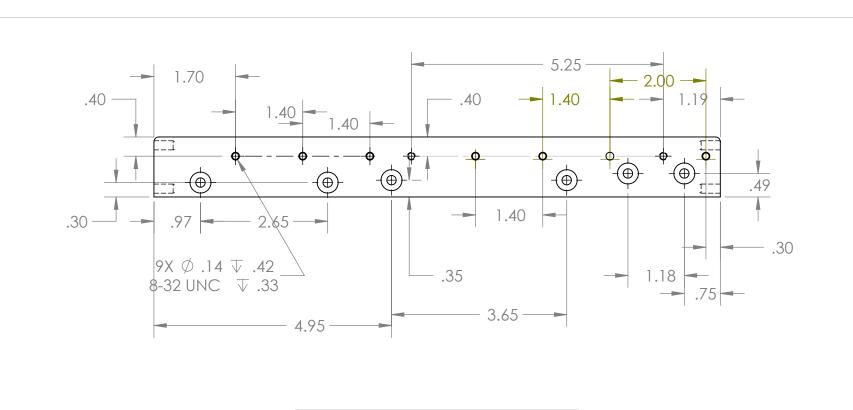
26	CS-52	4-40 "Silver-Grip" Set Screw	PIC design	10	http://www.pic-design.com/
27	B11-18	Sintered Bronze Flanged Bearing, 0.377 bore, 0.375 length	PIC design	4	
28	AIOL-6-2.80-0001	3/8" diameter, C-1060, PRECISION CASE HARDENED & GROUND	PIC design	4	Inexpensive custom part
29	4621	#8-32 416 Stainless Steel SOCKET HEAD SHOULDER SCREW, B=5/8"	PIC design	6	
30	Z1-2	retaining ring	PIC design	6	
31	Z1-1	retaining ring	PIC design	6	
32	4WDX4	LIFT-OFF HINGE, POLISHED, 1.97 X 1.26 IN, Left	THE OFFICE GROUP INC , http://www.theofficestore.c	2	
33	38DZ57	Socket Head Cap Screw A2 Stainless Steel, M5 Thread Dia., 45mm Length under Head, pkg/50	THE OFFICE GROUP INC	1	
34	2WB54	Socket Head Cap Screw, 18-8 Stainless Steel, #4 Thread Dia., 1/2" Length, pkg. 100	THE OFFICE GROUP INC	1	
35	5RVN5	Socket Head Cap Screw, Black Oxide Finish, 10-32 thread, 3/8 Thread Length, pkg/100	THE OFFICE GROUP INC	1	
36	22TU96	SHCS Standard 6-32x1/2 316 SS PK100 Socket Head Cap Screw, pkg/100	THE OFFICE GROUP INC	1	
37	22TU87	SHCS Standard 6-32x1/2 316 SS PK100 Socket Head Cap Screw, pkg/100	THE OFFICE GROUP INC	1	
38	3ZMV5	Round Standoff Female Nylon Screw Size #10-32 Outside Dia. 1/2 In Length , pkg/100	THE OFFICE GROUP INC	1	
39	6UKX1	Flat Washer Standard 316 Stainless Steel Finish Plain Fits Bolt Sizes #10, pkg/100	THE OFFICE GROUP INC	1	
40	2DNY1	Flat Washer Standard 316 Stainless Steel Finish Plain Fits Bolt Sizes 4, pkg/25	THE OFFICE GROUP INC	1	
41	2DNY2	Flat Washer Standard 316 Stainless Steel Finish Plain Fits Bolt Sizes 6, pkg/25	THE OFFICE GROUP INC	1	
42	C82VX-1676EH6	6-port Valco Cheminert valve, one vertical port, 15000 psi, 250 $\mu$ m bore, 1/16" Fittings with EH series Microelectric Actuator and 6" Standoff Assembly	VICI Valco Instruments, www.vici.com	1	Valve #1
43	C82X-1670DEH6	10-port Valco Cheminert valve, 15000 psi, 250 µm bore, 1/16" Fittings with EH series Microelectric Actuator and 6" Standoff Assembly	VICI Valco Instruments, www.vici.com	1	Valve #4
44	C82X-1676EH6	6-port Valco Cheminert valve, 15000 psi, 250 μm bore, 1/16"" Fittings with EH series Microelectric Actuator and 6" Standoff Assembly	VICI Valco Instruments, www.vici.com	1	Valve #2
45	C82U-1676EH6-NIST	6-port Valco Cheminert valve, 22000 psi, 1/16" Fittings with EH series Microelectric Actuator and 6" Standoff Assembly	VICI Valco Instruments, www.vici.com	1	Valve #3
46	2020 part cut 7.87"	strut 20mm x 20mm x 7.95in long, Tap M5 both ends, 6061	0000	3	0000
47	long 473-1122-ND	Aluminun Grease Silver Conductive (MG Chemicals 8463-7G), 0.25 oz	8020, Inc Digikey	1	<u>www.8020.net</u>



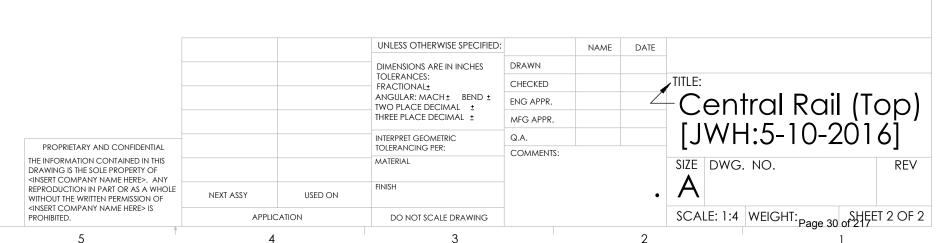




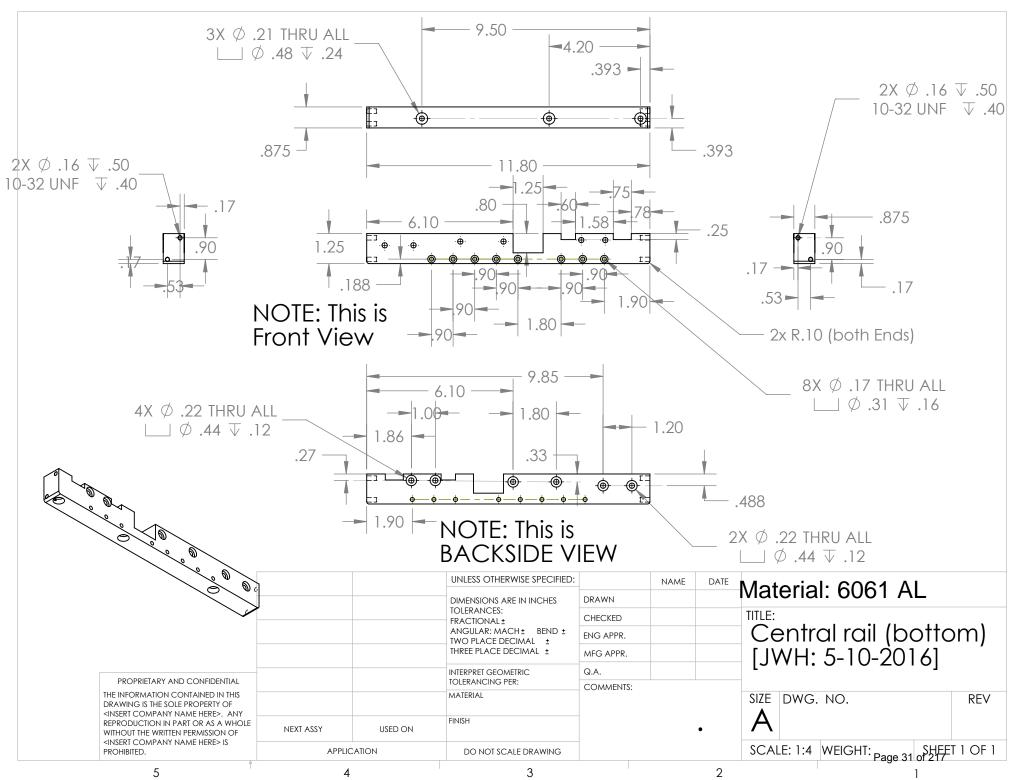


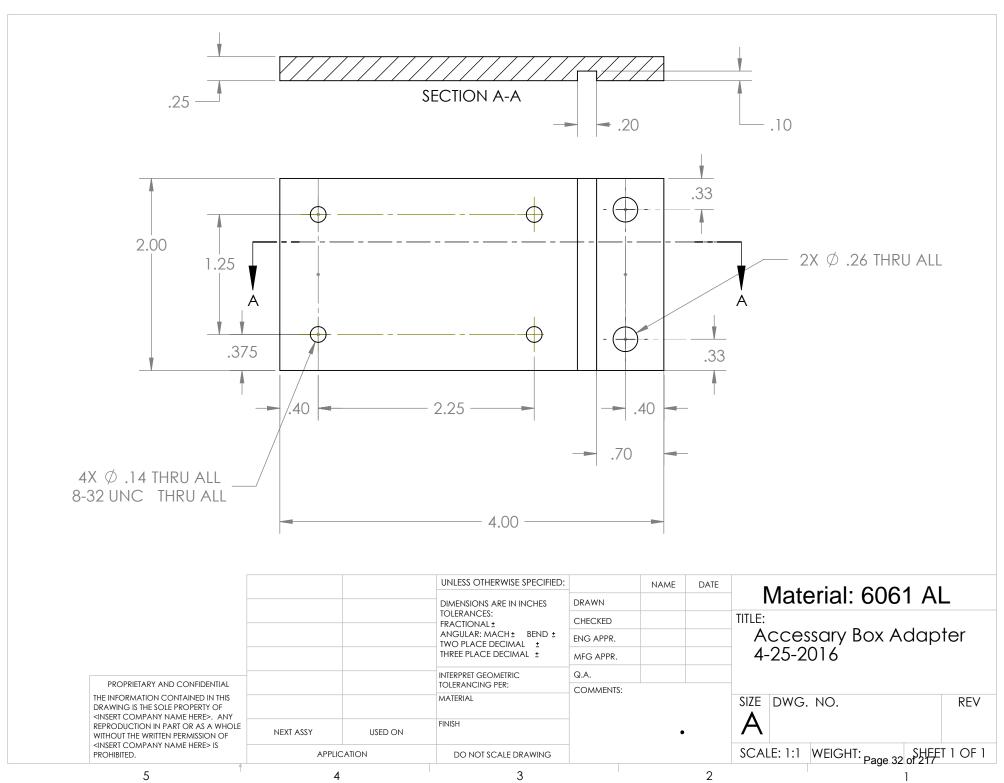


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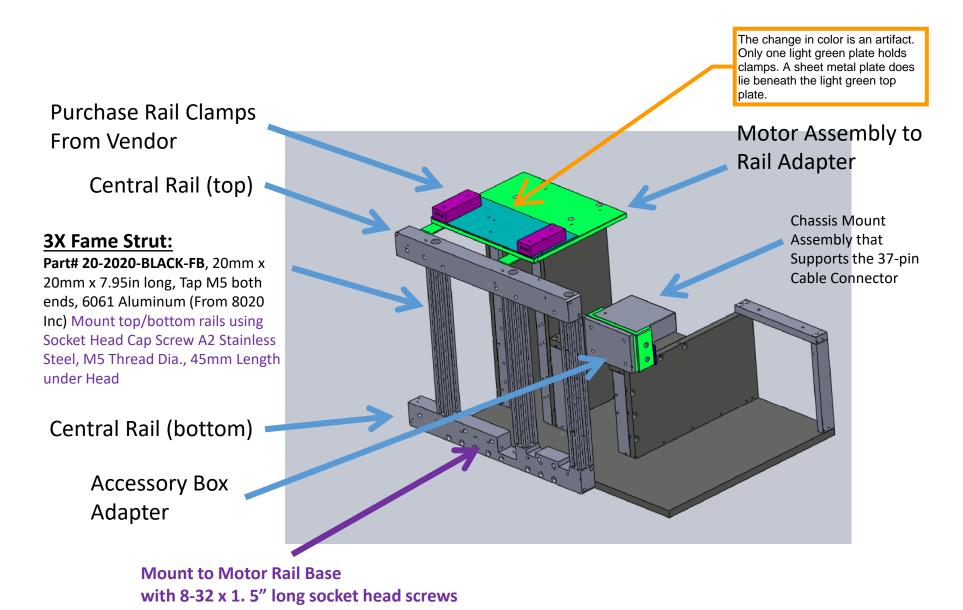


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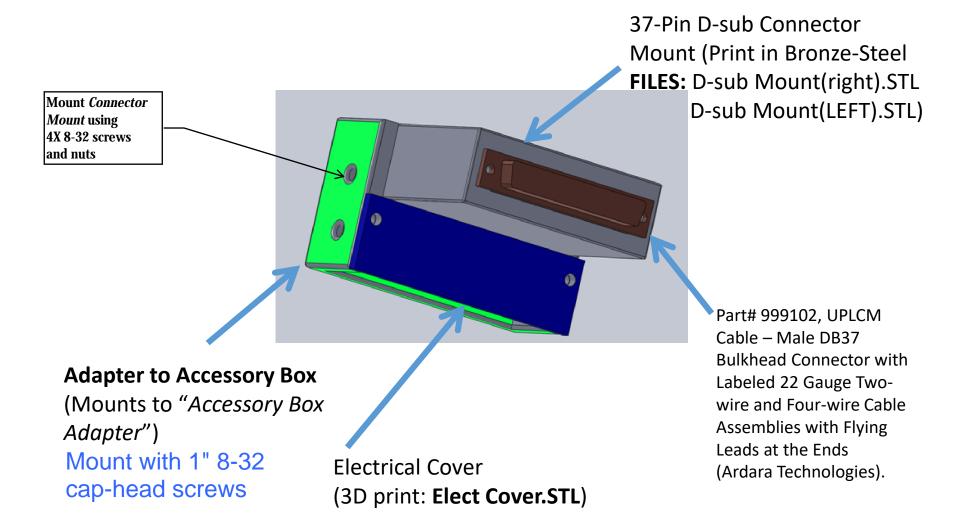


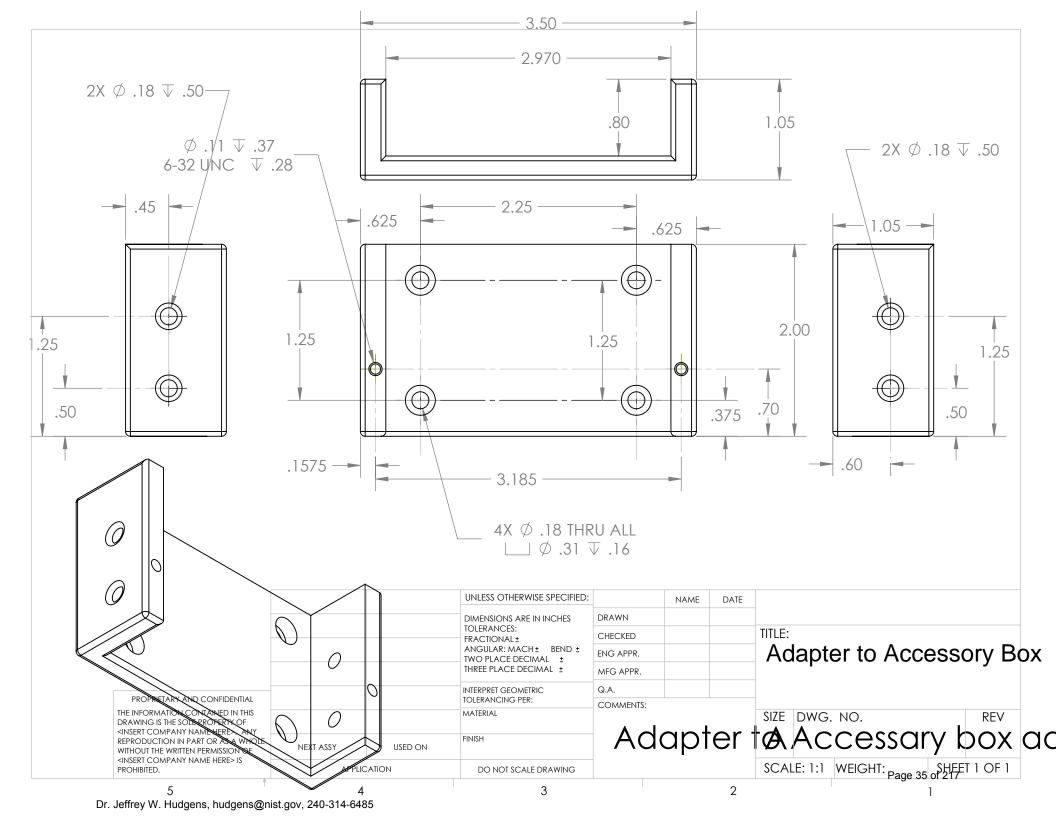


#### Overview of Metal Frame Assembly

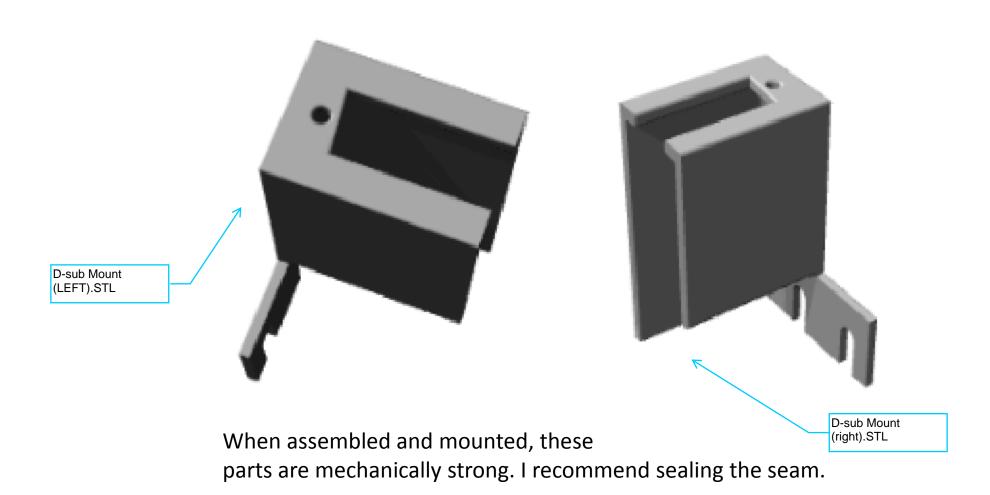


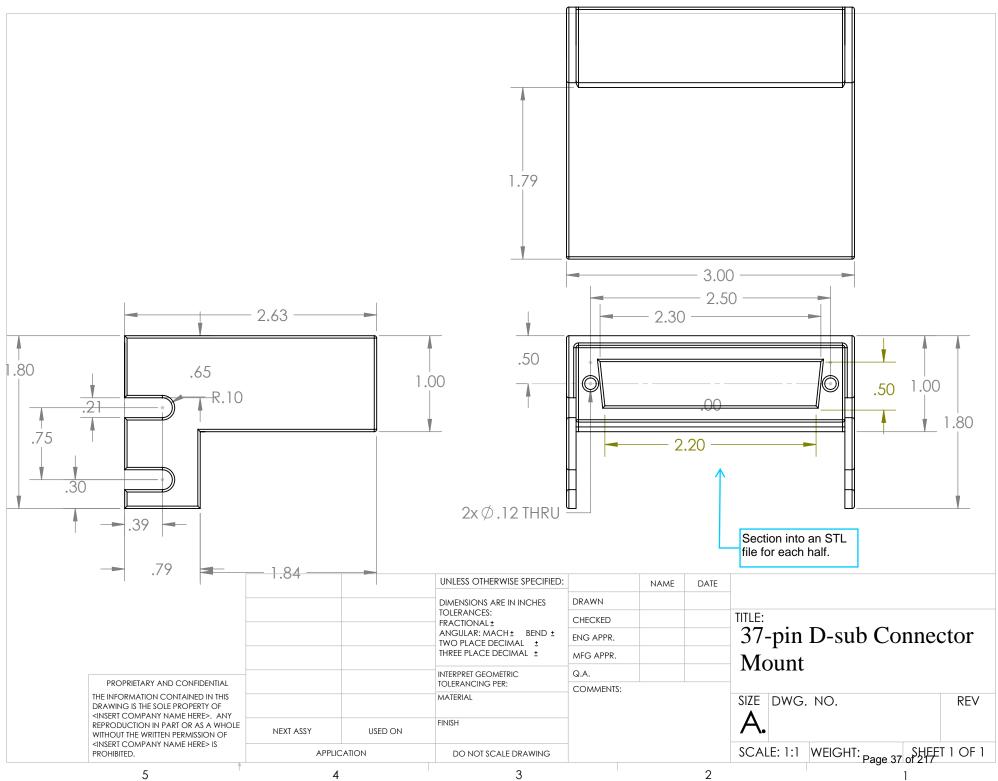
# Chassis Mount Assembly that Supports the 37-pin Cable Connector

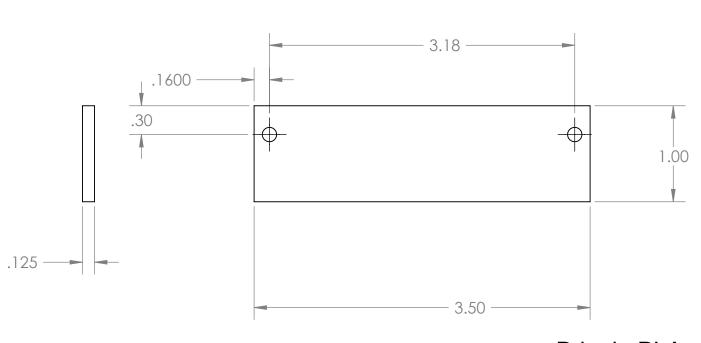




## 37-pin D-sub Connector Mount



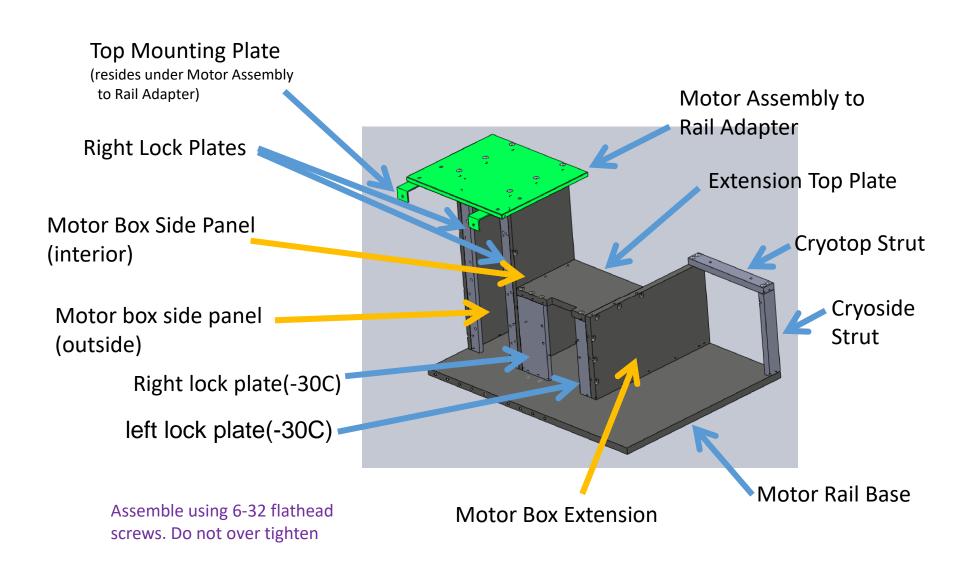


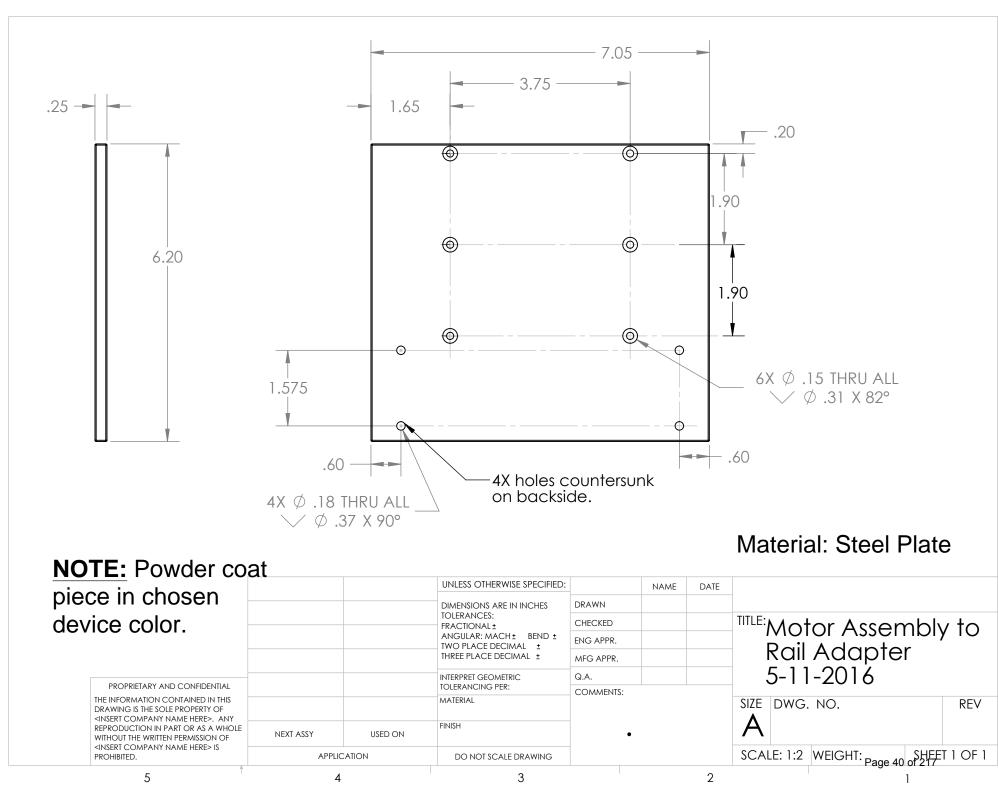


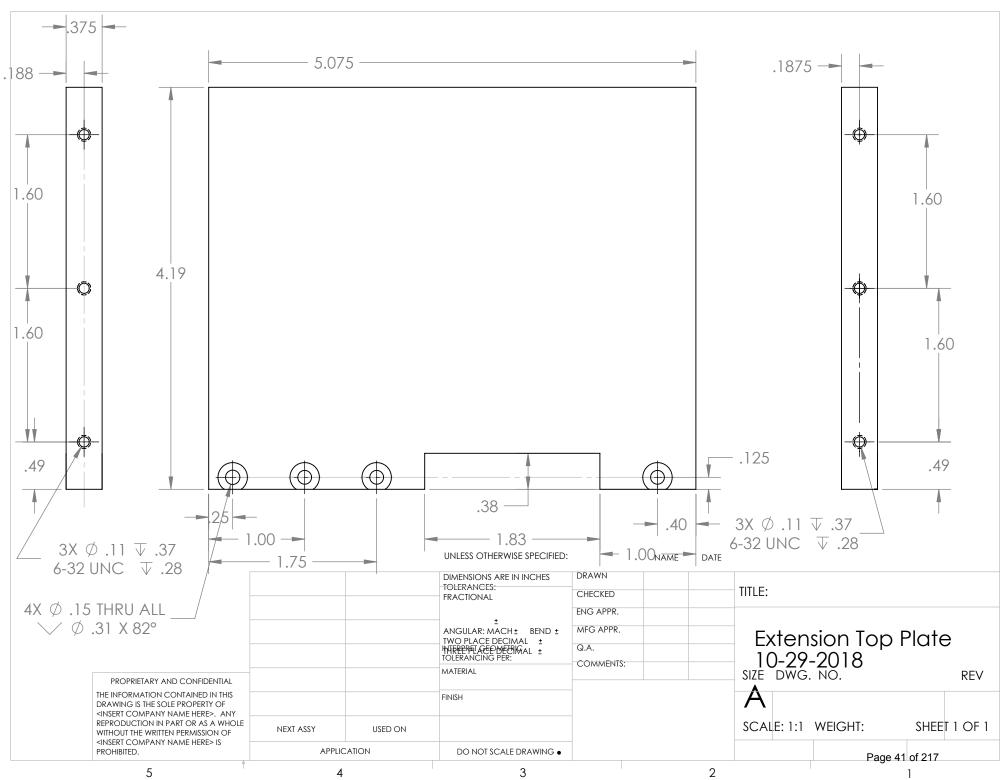
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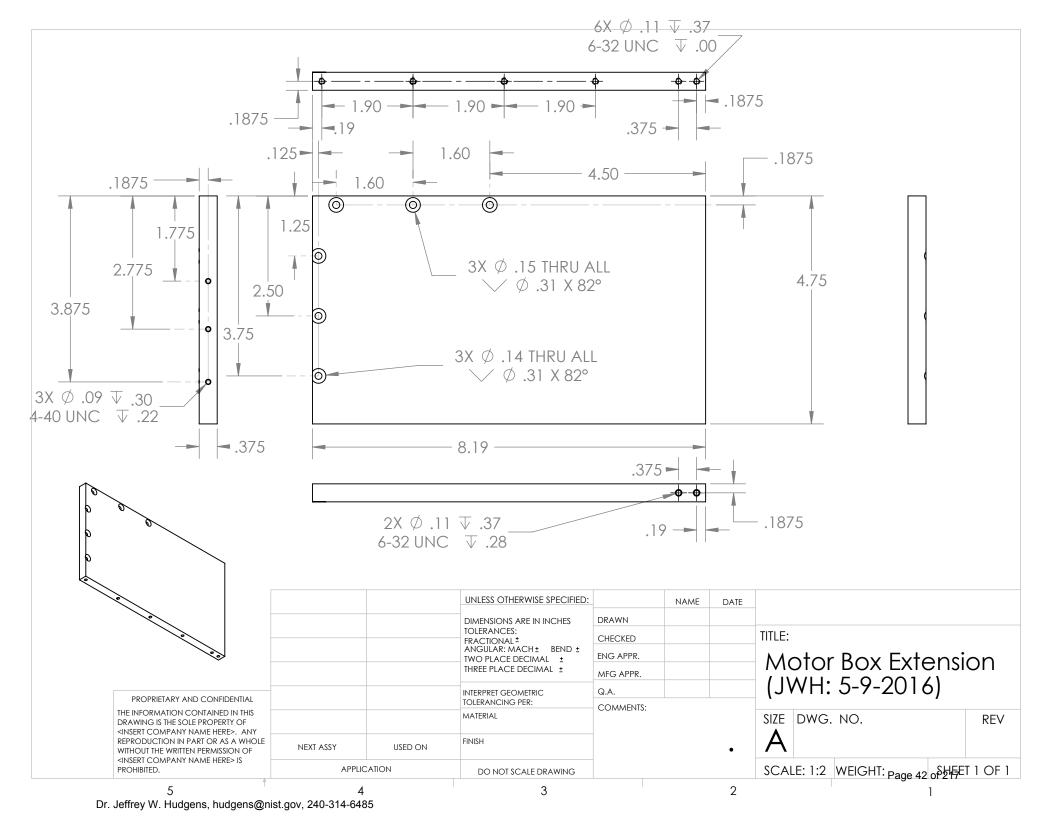
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			DIMENSIONS ARE IN INCHES	DRAWN				
_			TOLERANCES: FRACTIONAL± ANGULAR: MACH± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ± INTERPRET GEOMETRIC TOLERANCING PER: MATERIAL	CHECKED			TITLE: Electrical Cover	
				ENG APPR.				
				MFG APPR.			Electrical Cover	
PROPRIETARY AND CONFIDENTIAL				Q.A.				
THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF <insert company="" here="" name="">. ANY REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF <insert company="" here="" name=""> IS PROHIBITED.</insert></insert>				COMMENTS:			SIZE DWG. NO. REV	
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	APPLICATION		DO NOT SCALE DRAWING				SCALE: 1:1 WEIGHT: Page 38 of 217	
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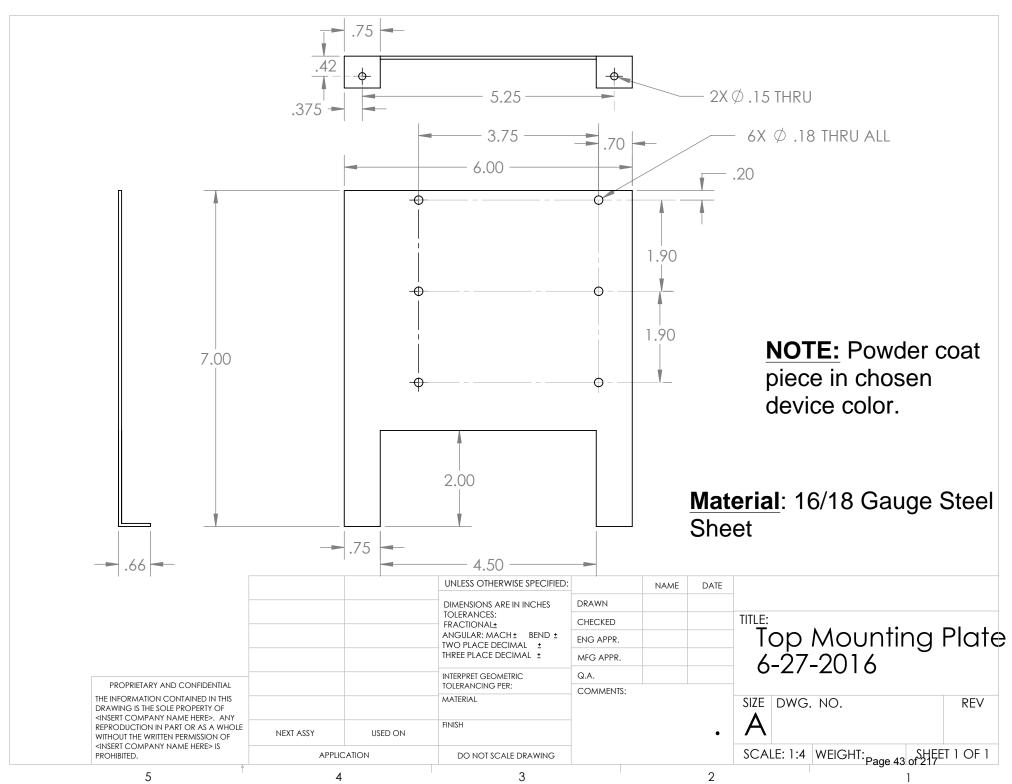
## Overview of Motor Hanger Assembly

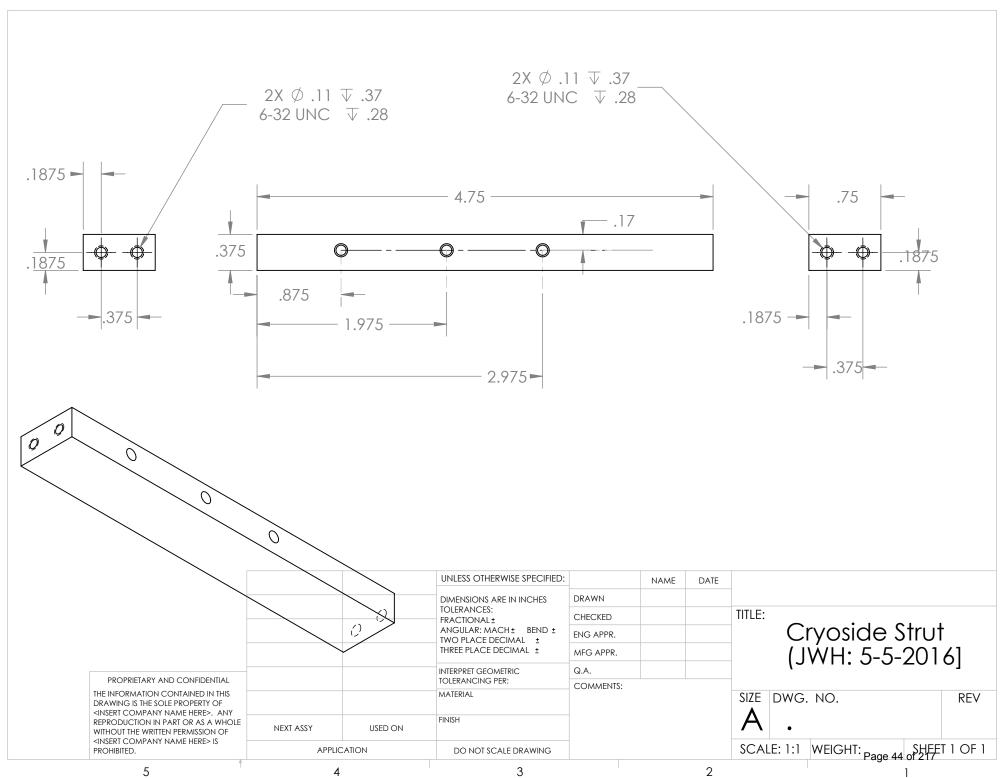


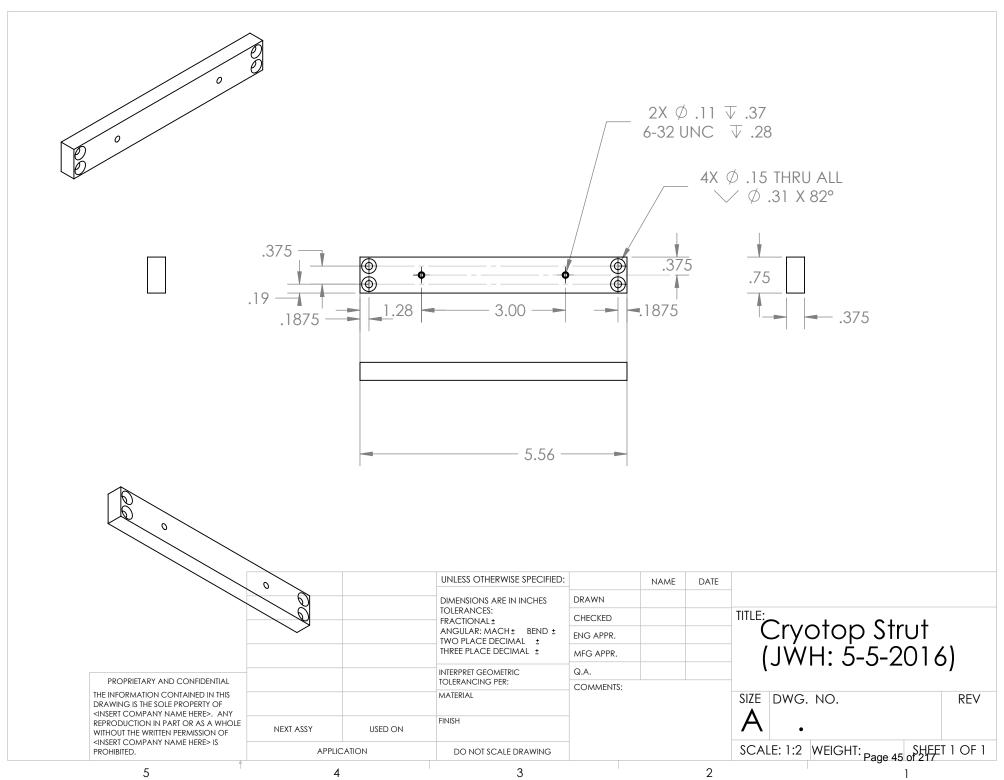


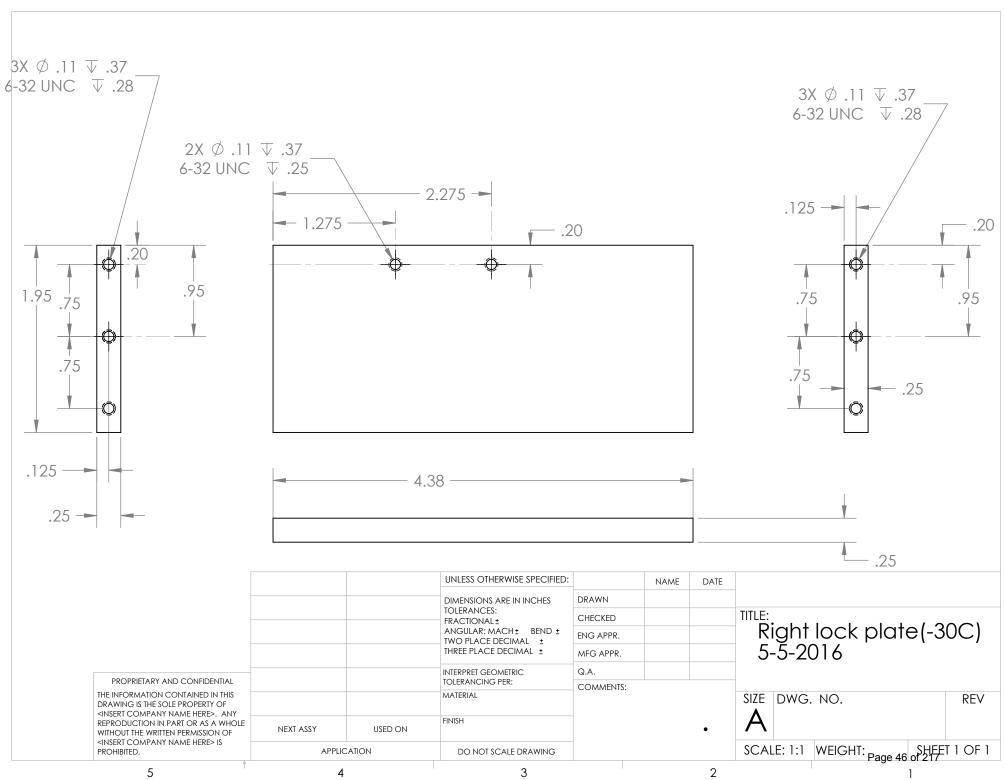


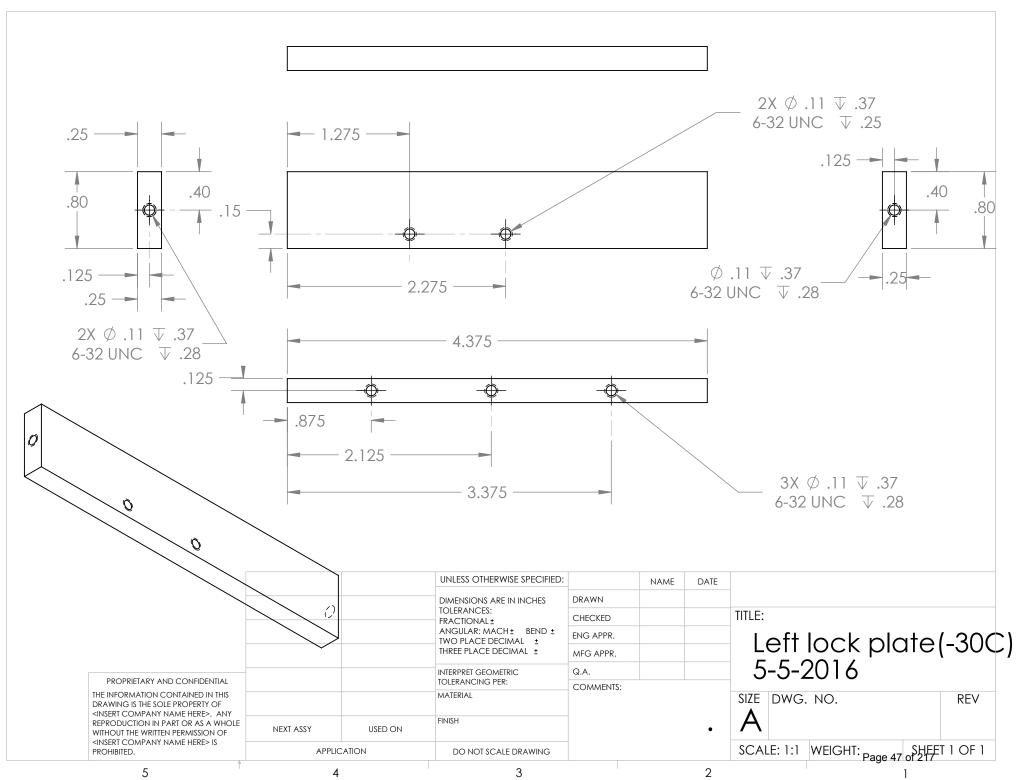


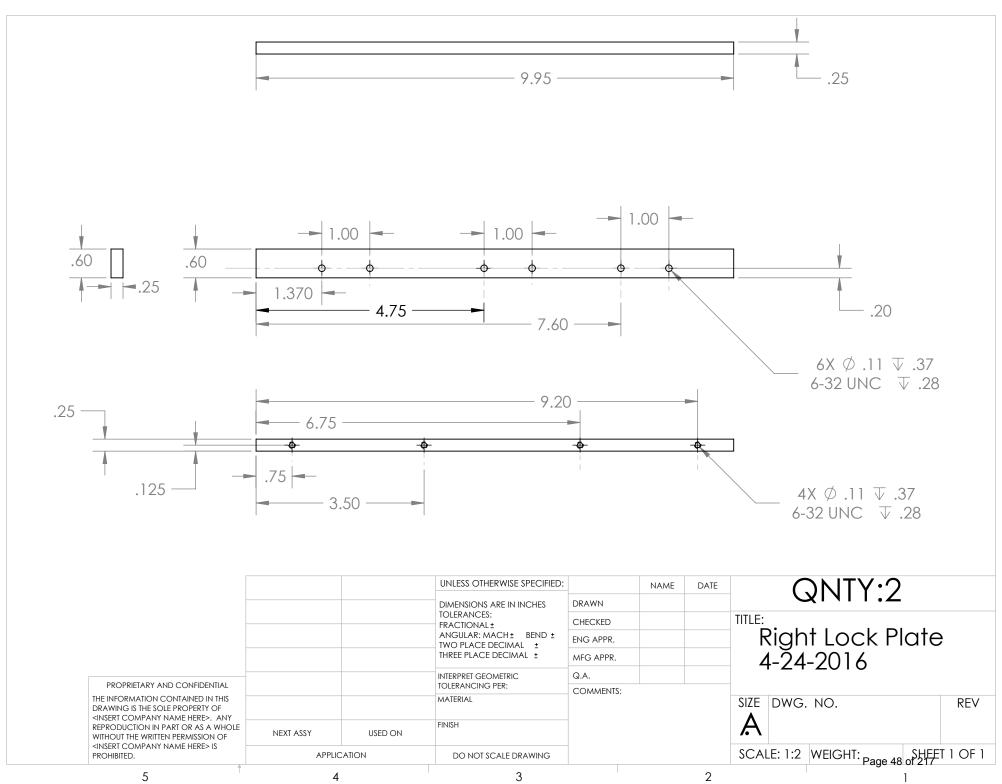


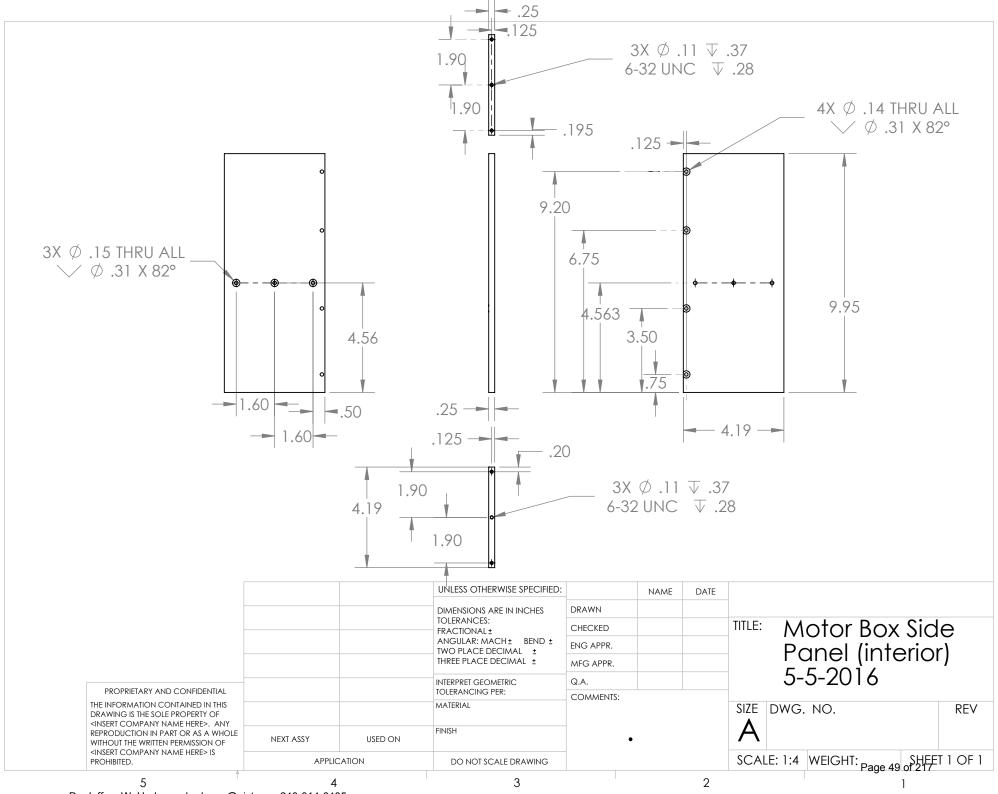


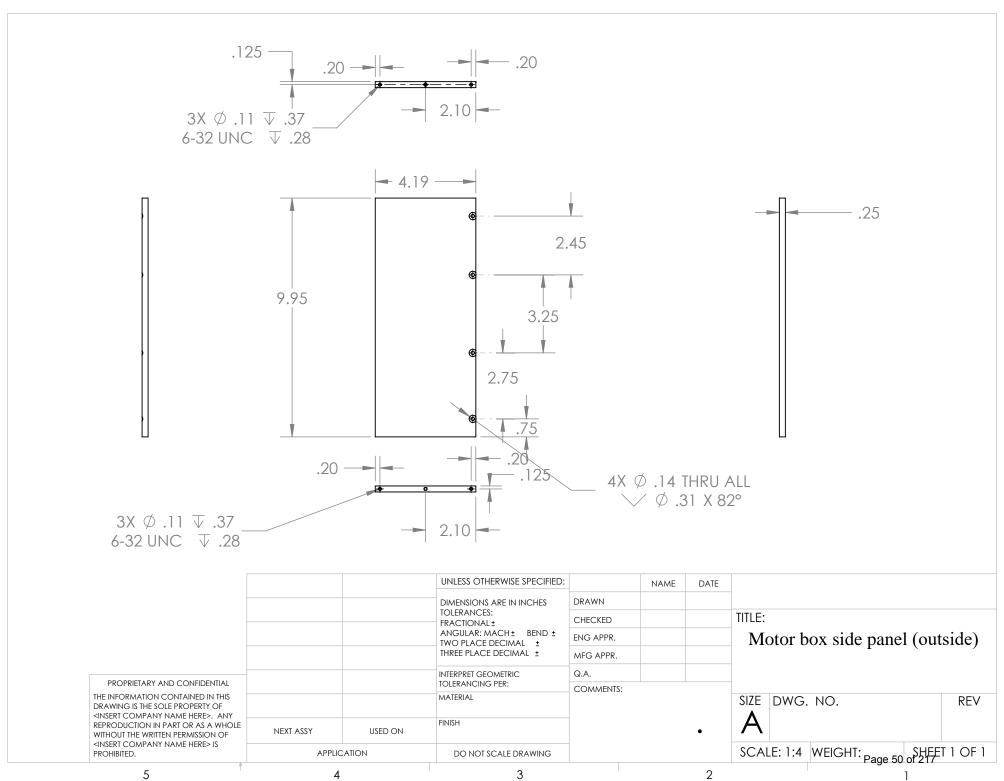


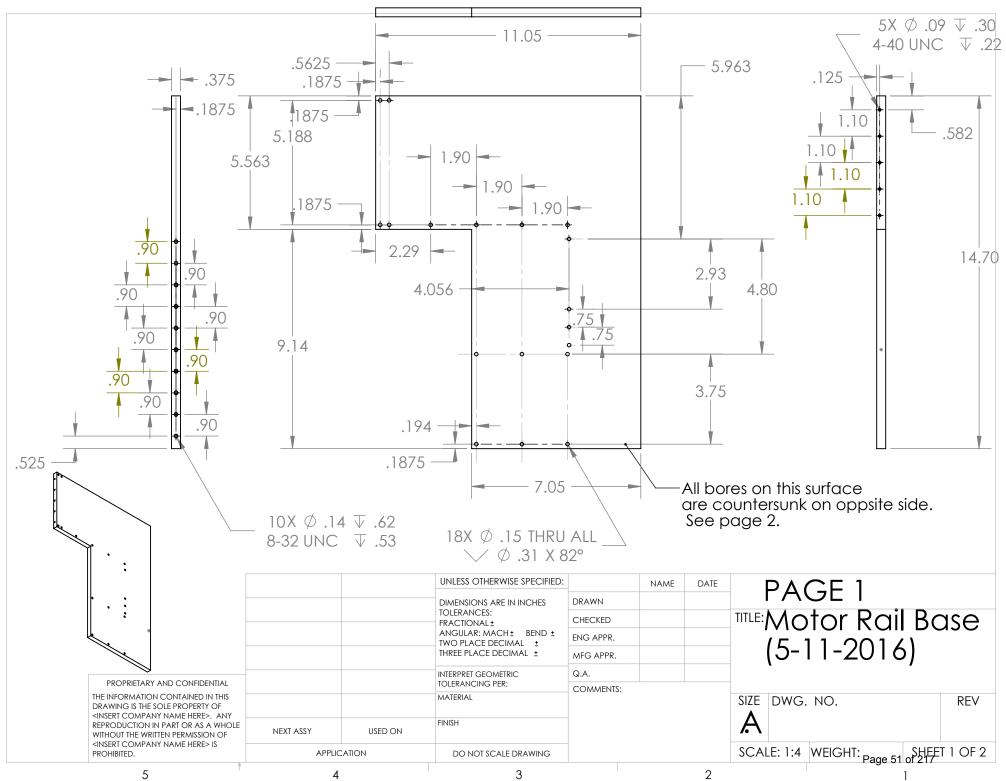


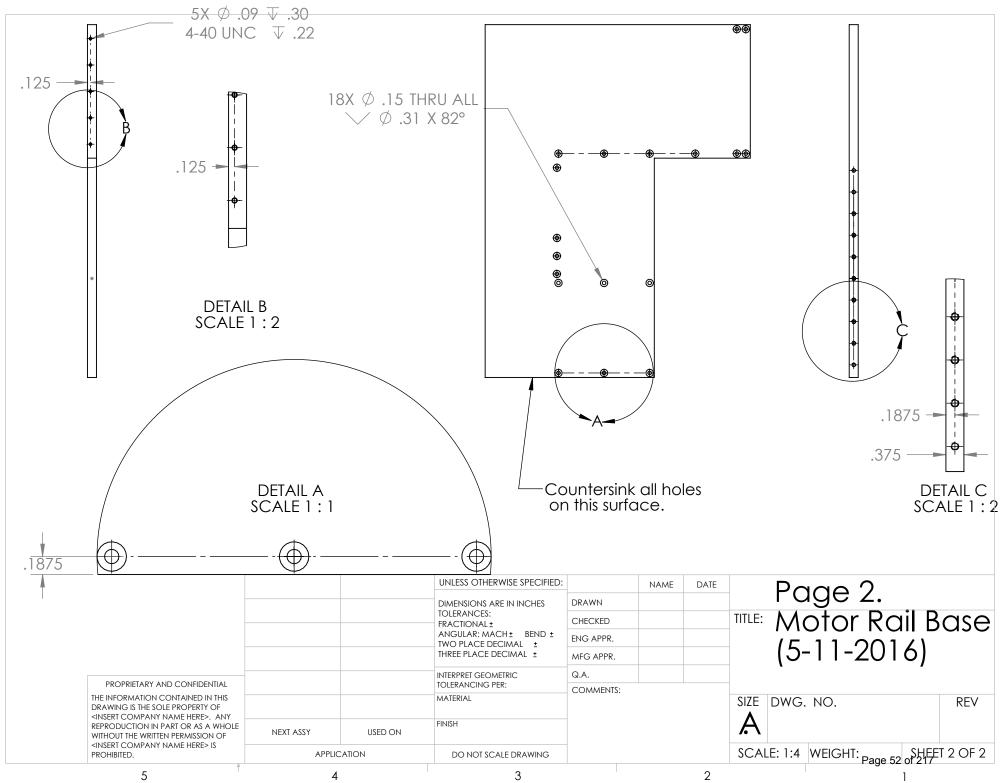




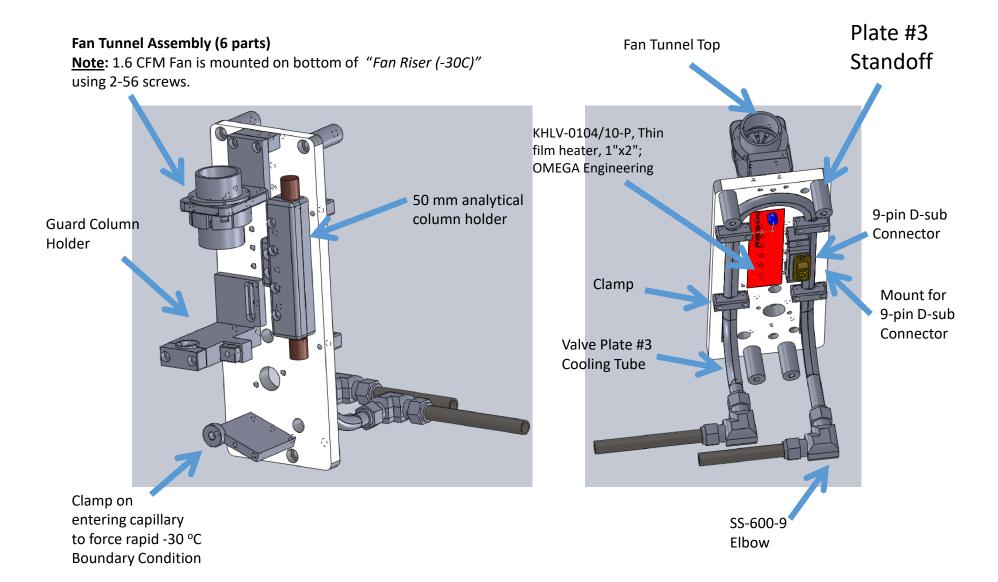


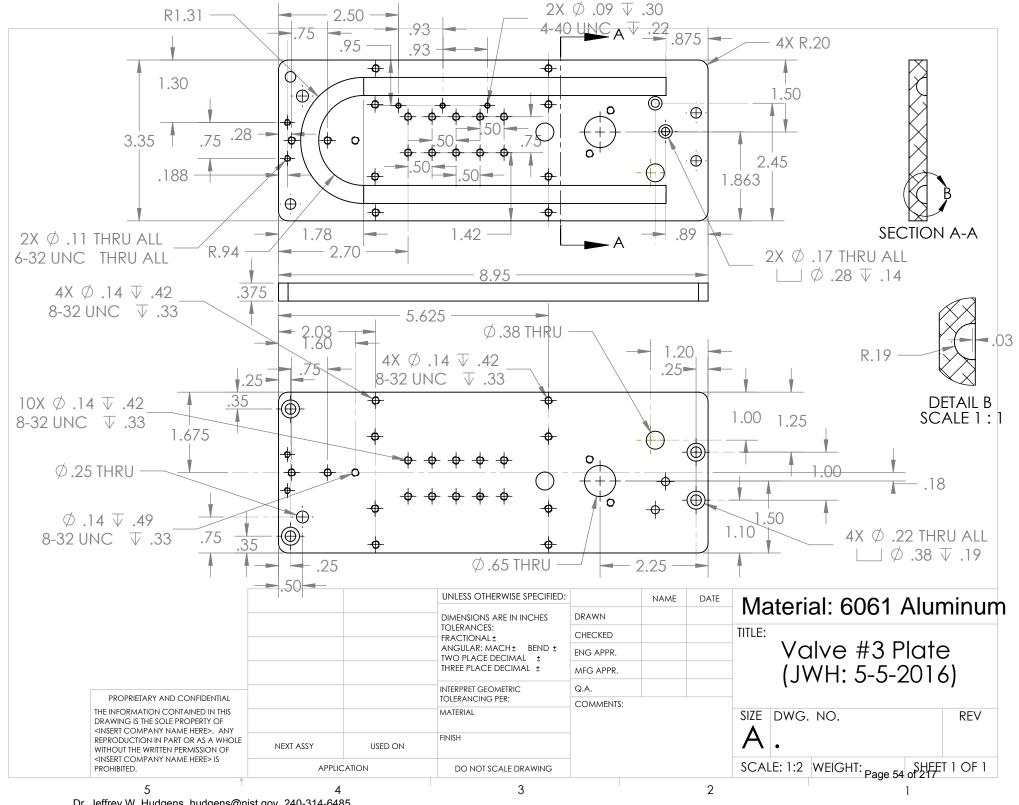


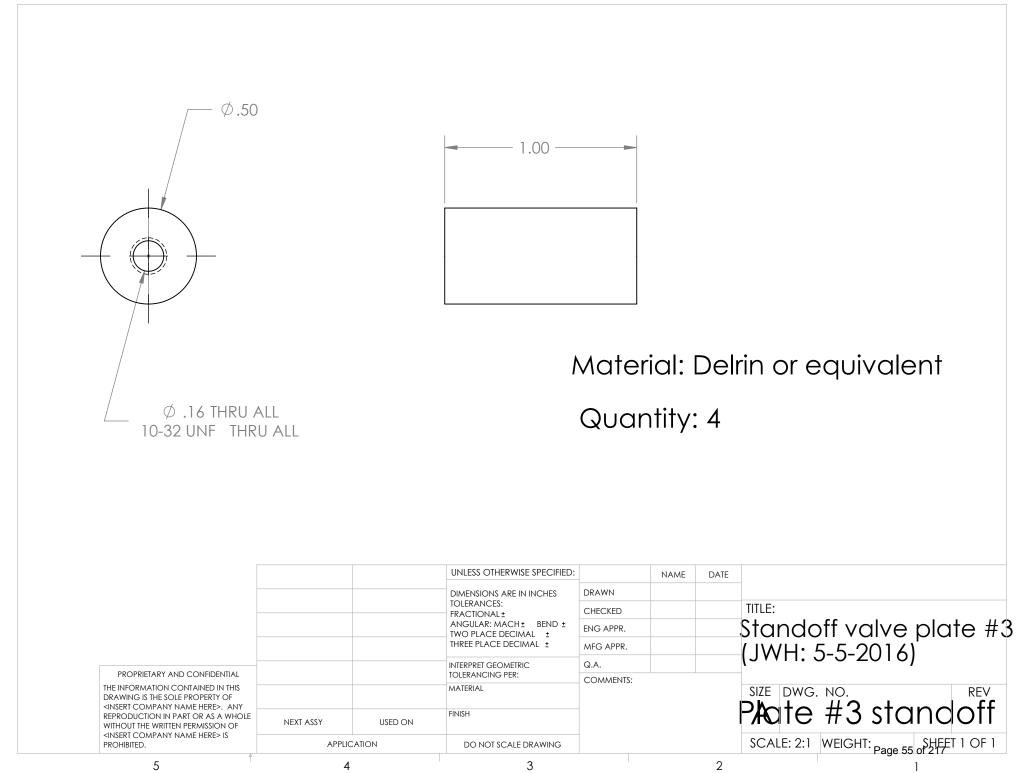


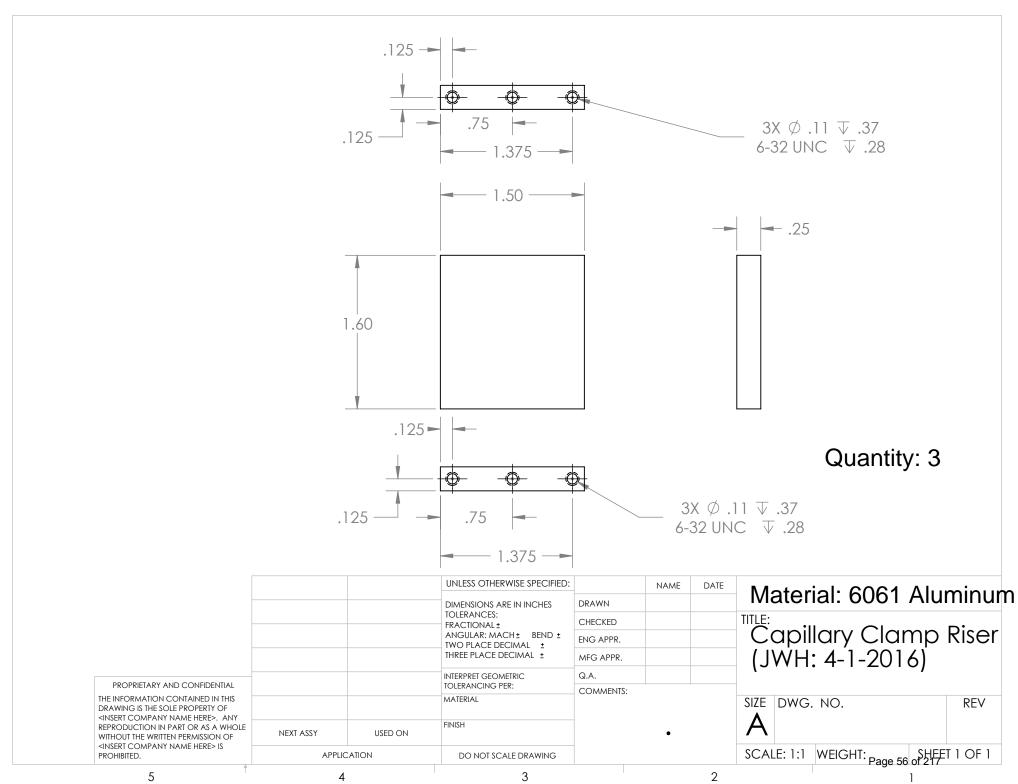


### Valve #3 Plate Assembly

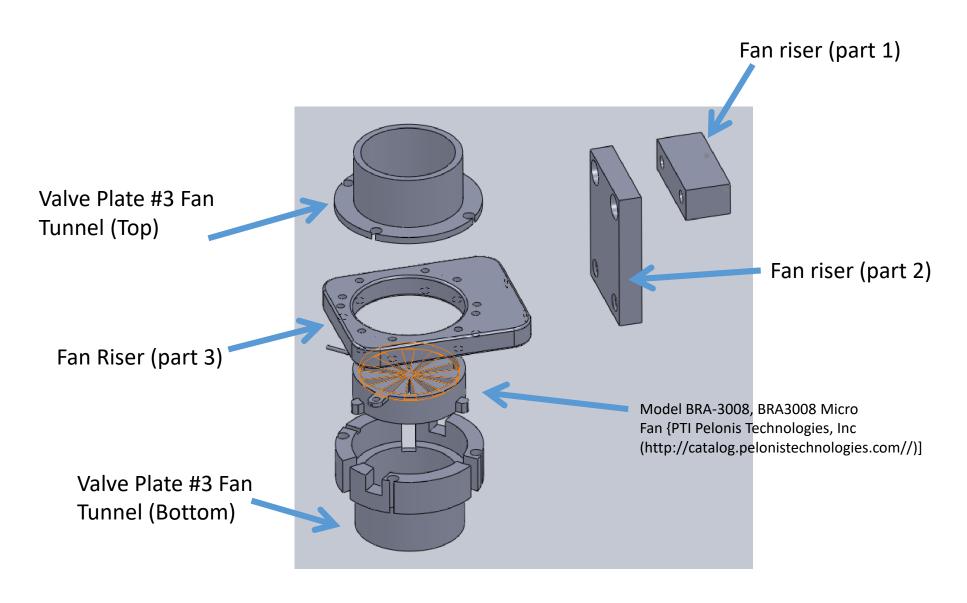


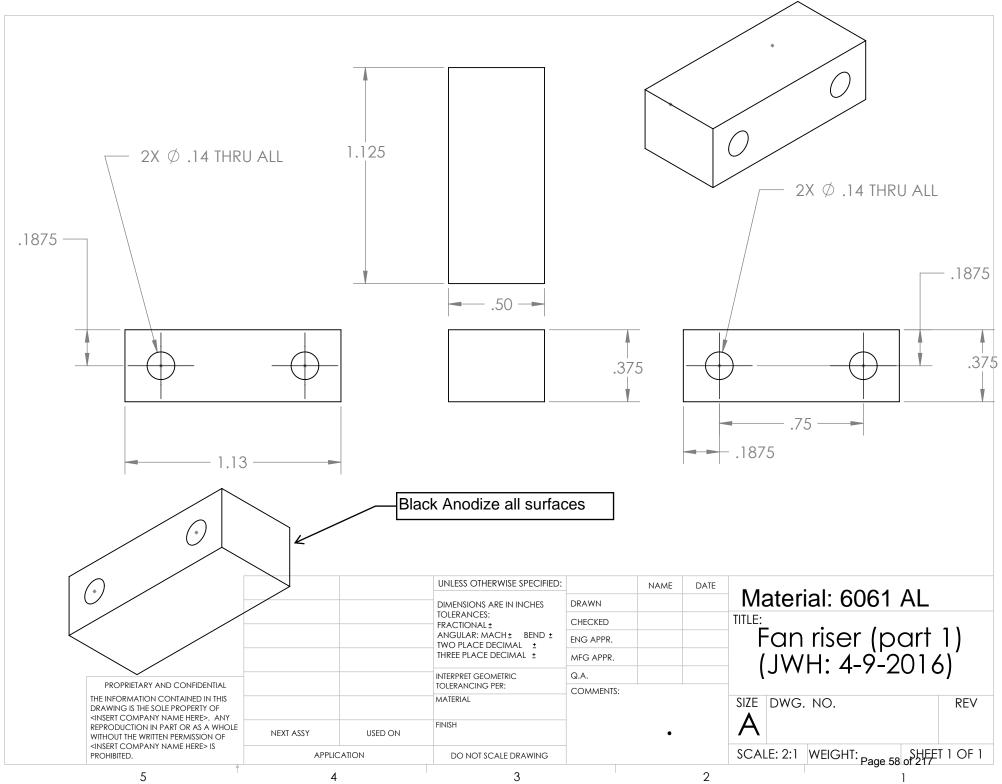


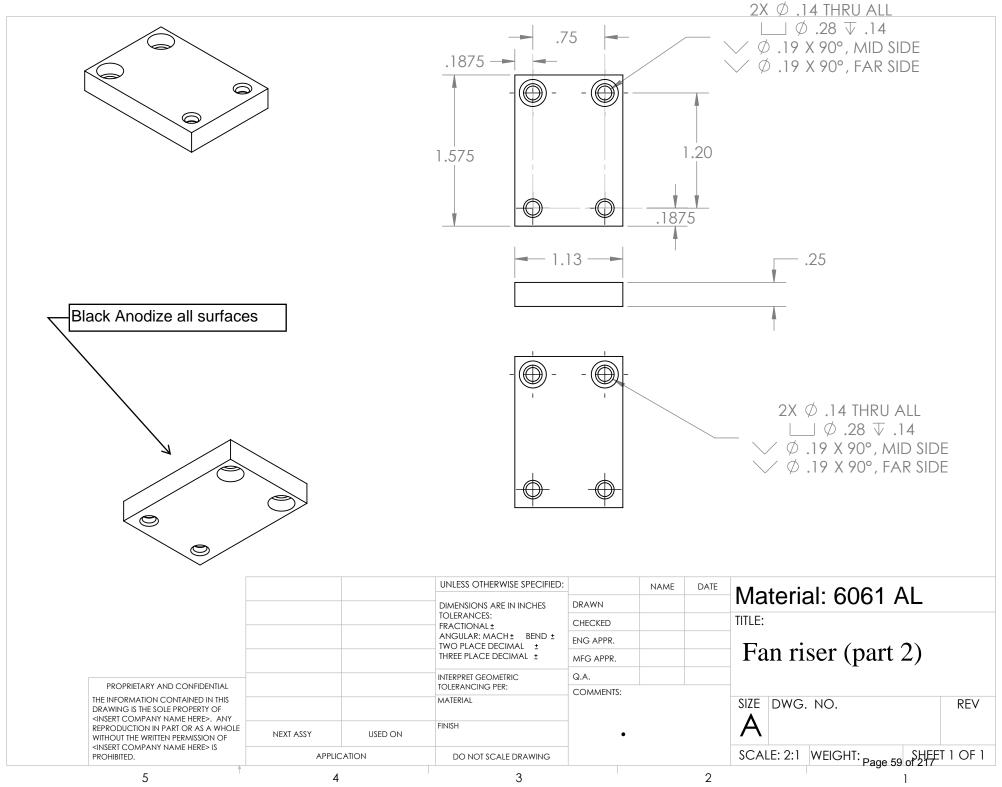


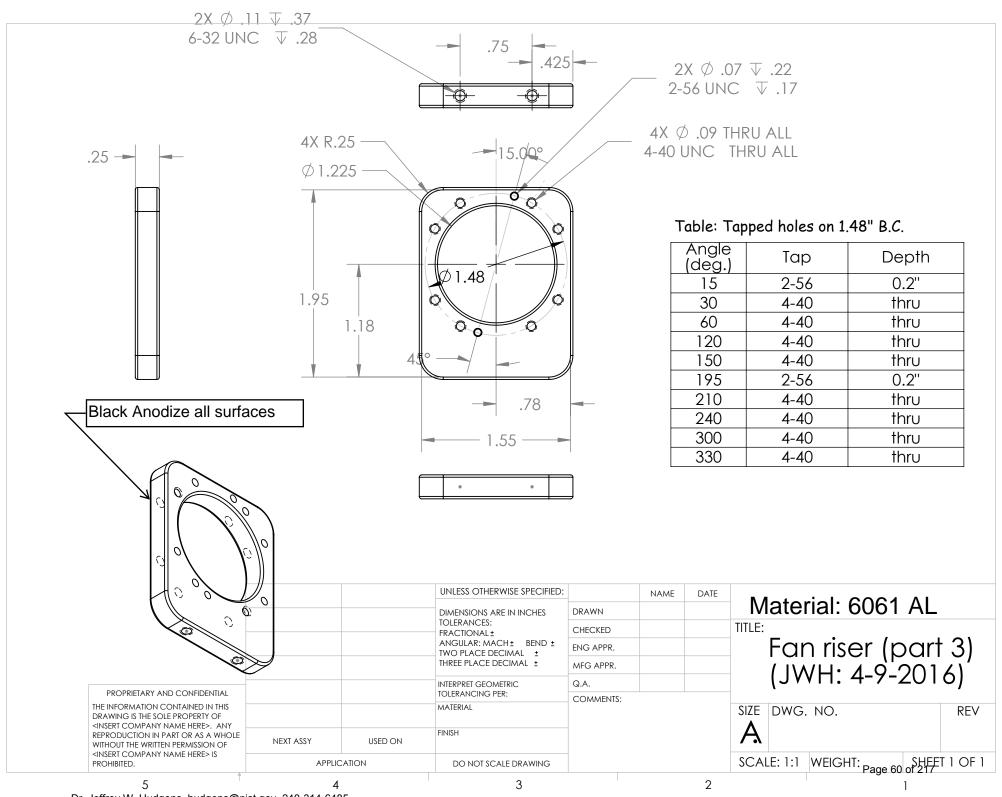


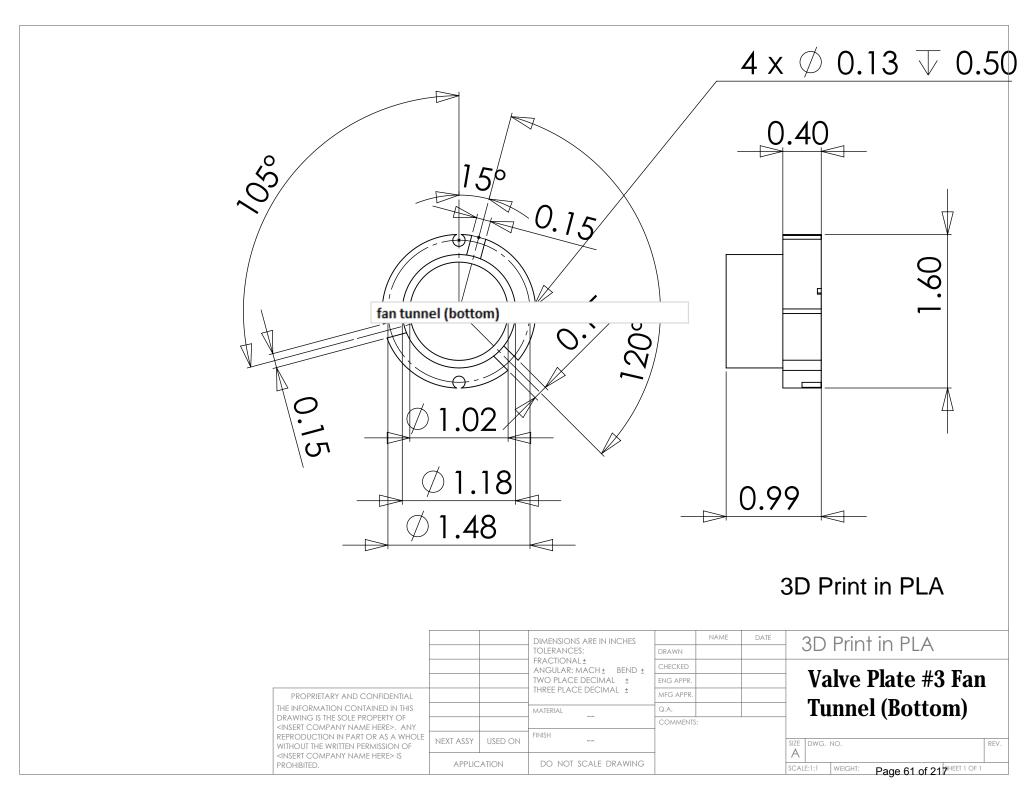
# Plate #3 Fan Assembly

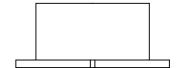


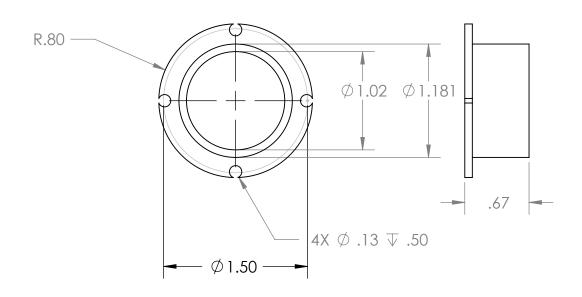












3D Print in PLA DIMENSIONS ARE IN INCHES TOLERANCES: DRAWN FRACTIONAL ± CHECKED Valve Plate #3 Fan ANGULAR: MACH± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ± ENG APPR. MFG APPR. **Tunnel (Top)** Q.A. MATERIAL COMMENTS: FINISH USED ON A fan tunggl top (130) NEXT ASSY REV.

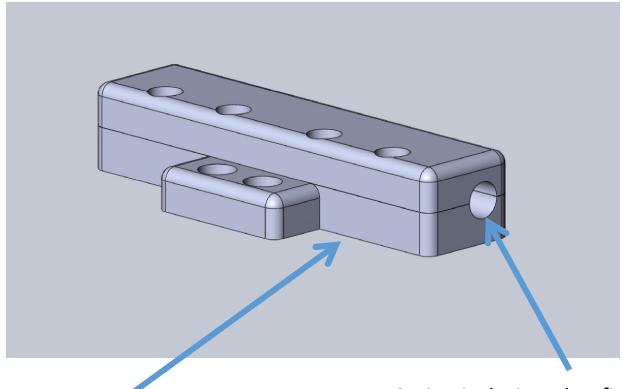
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APPLICATION

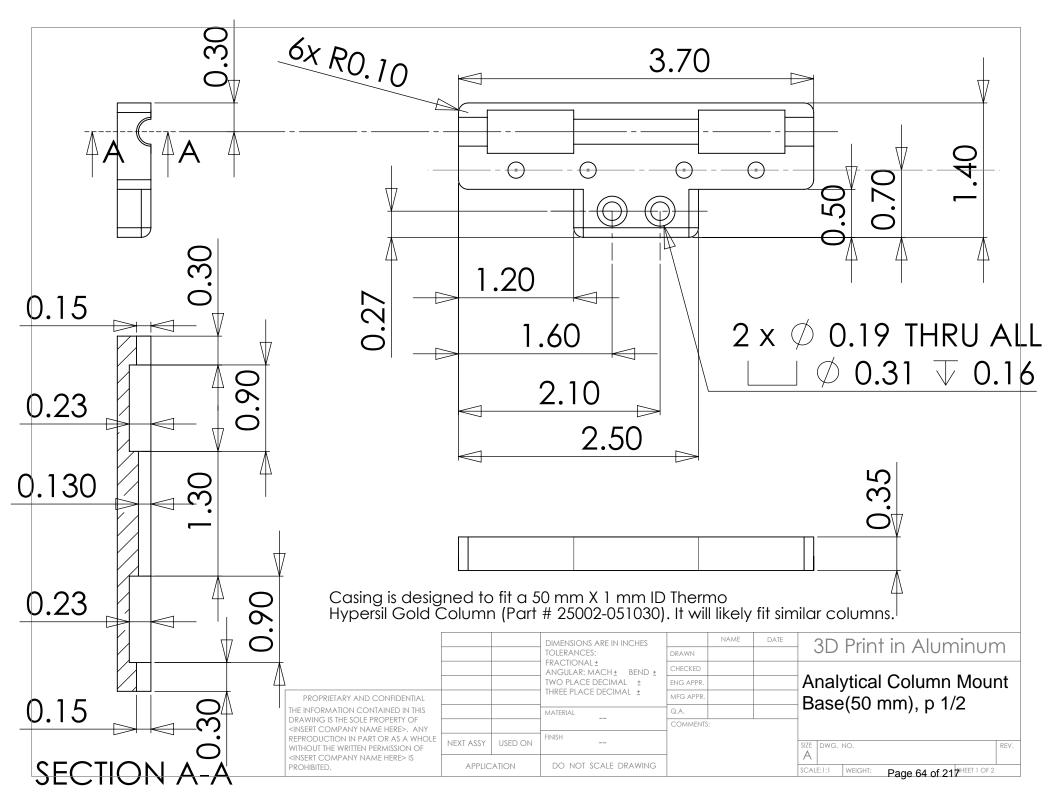
DO NOT SCALE DRAWING

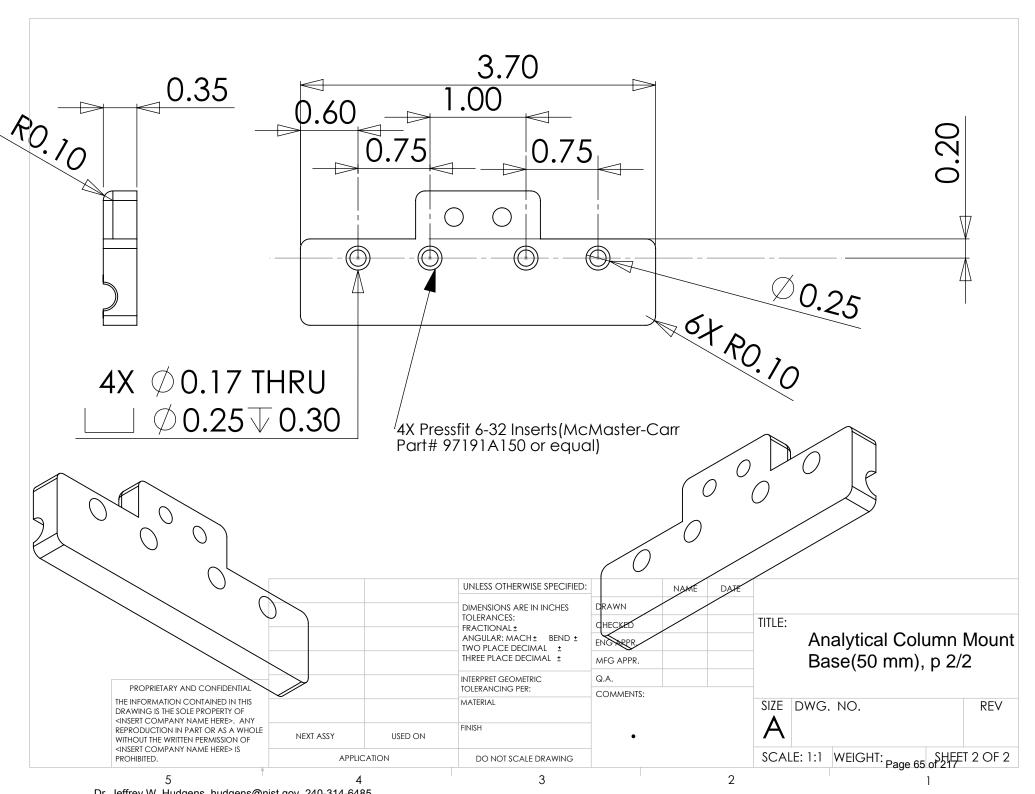
#### 50 mm LC Column Housing (Page 1 of 5)



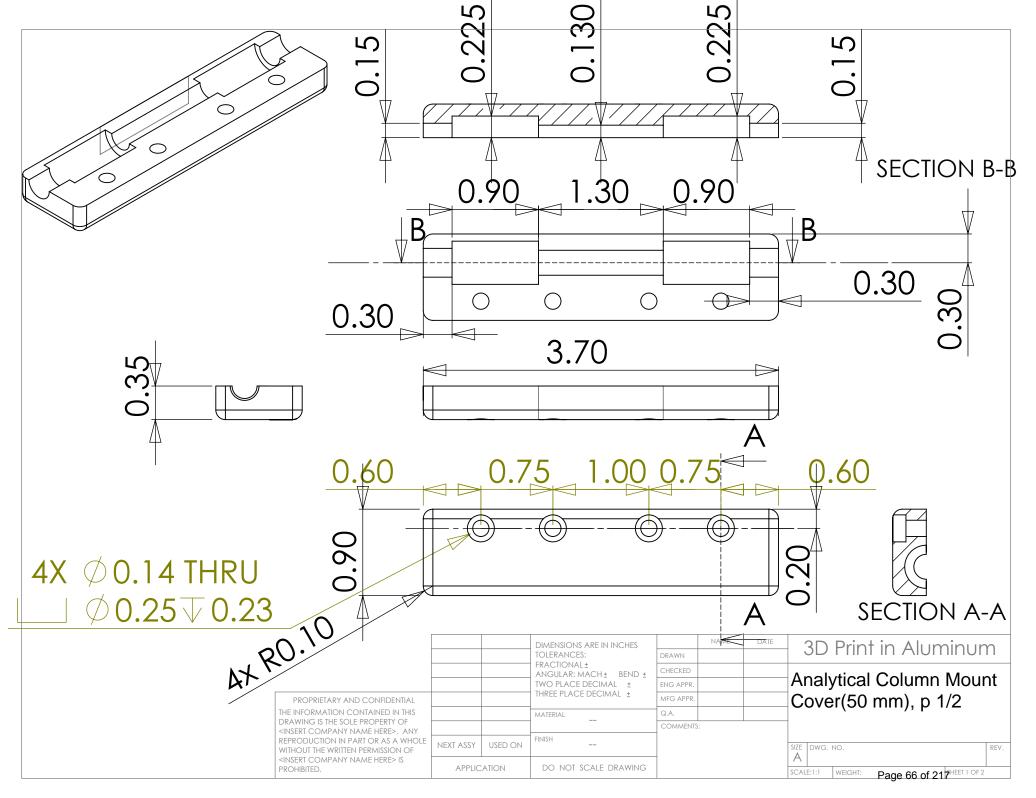
In hole insets on bottom, press-fit four 6-32 Inserts for Soft Metal (McMaster-Carr Part# 97191A150 or similar)

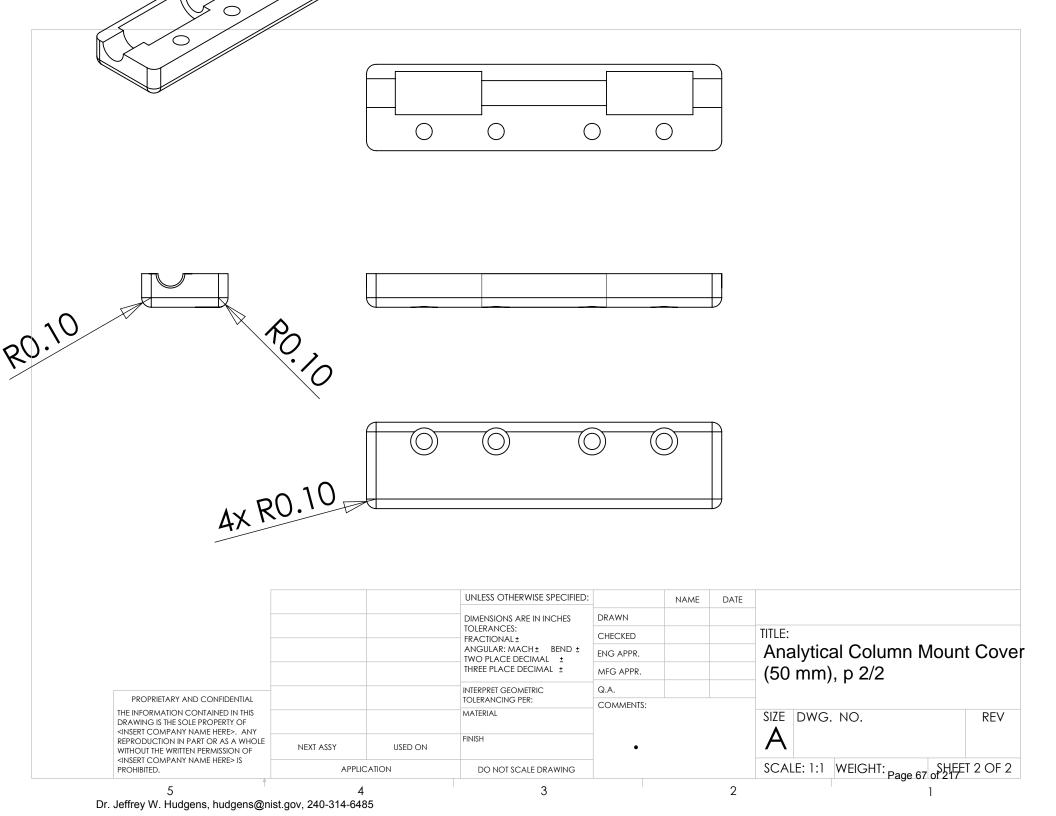
Casing is designed to fit a 50 mm X 1 mm ID Thermo Hypersil Gold Column (Part # 25002-051030). It will likely fit similar columns.

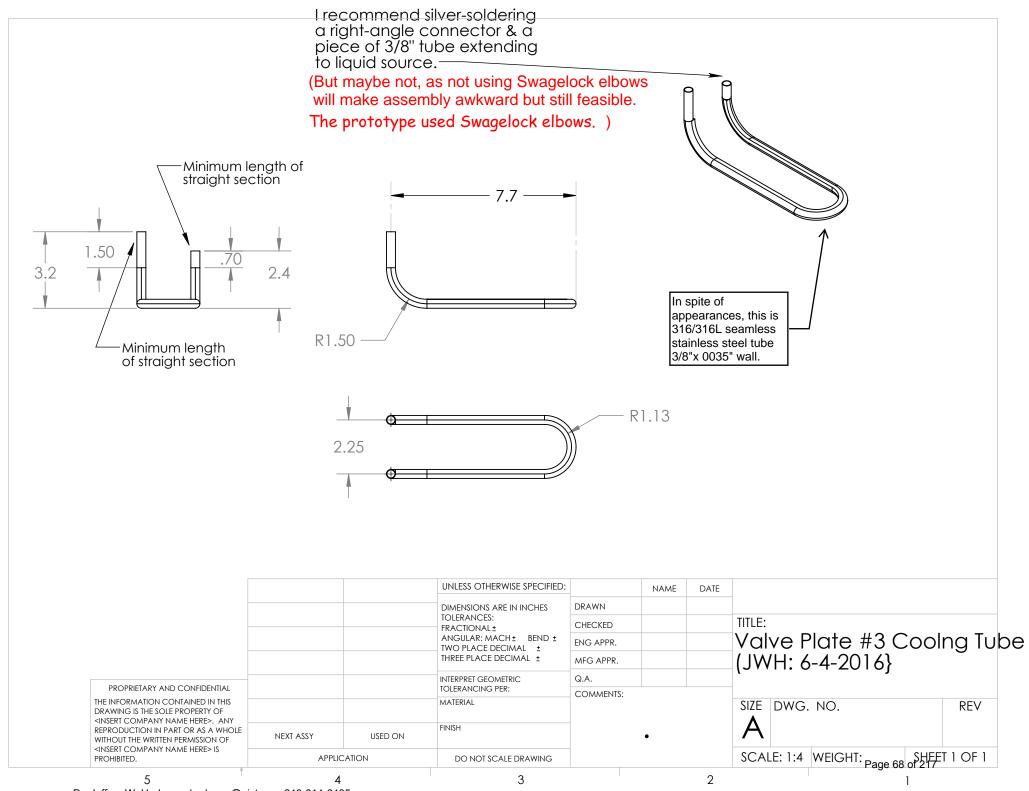


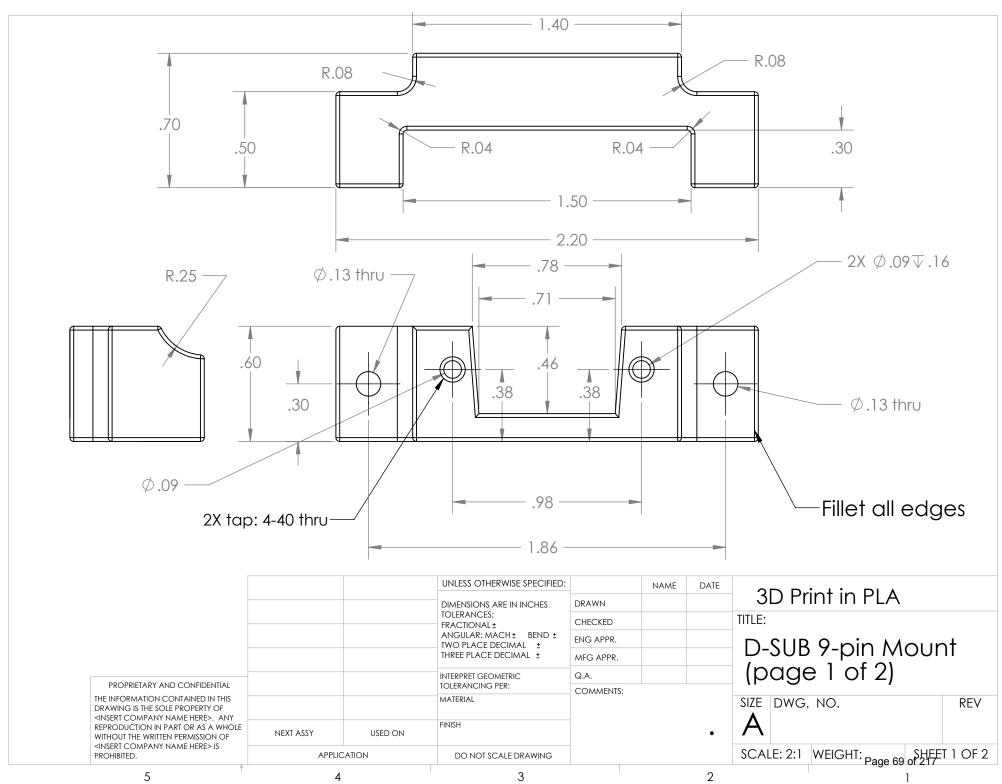


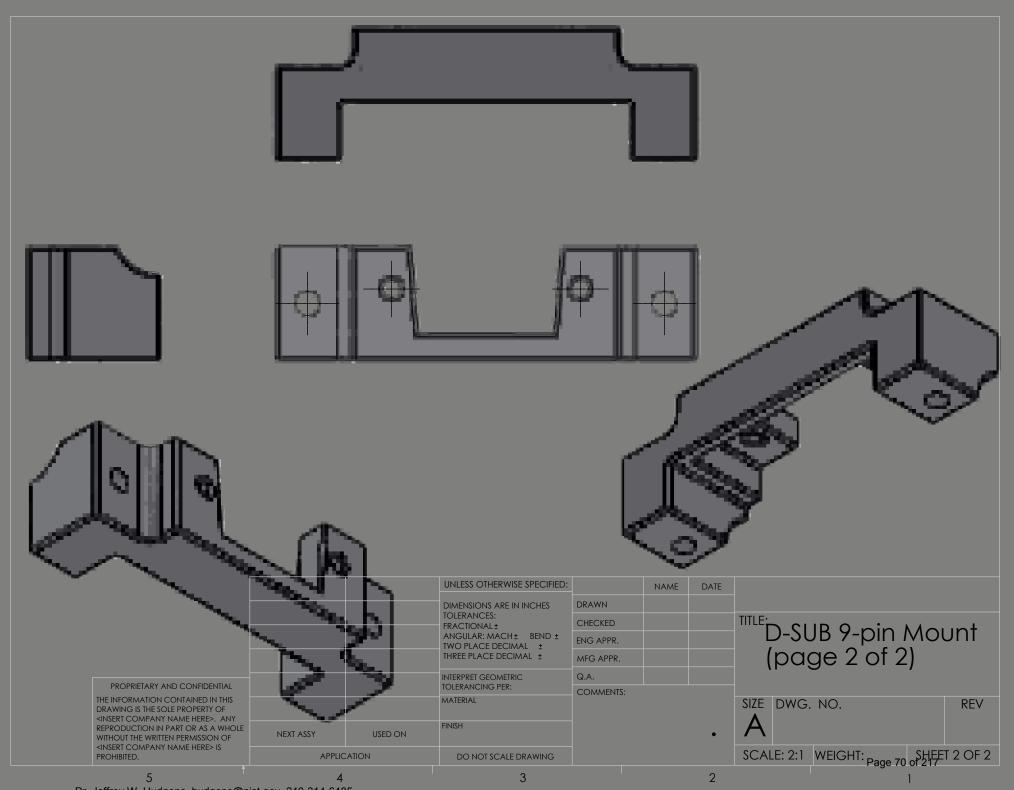
Dr. Jeffrey W. Hudgens, hudgens@nist.gov, 240-314-6485

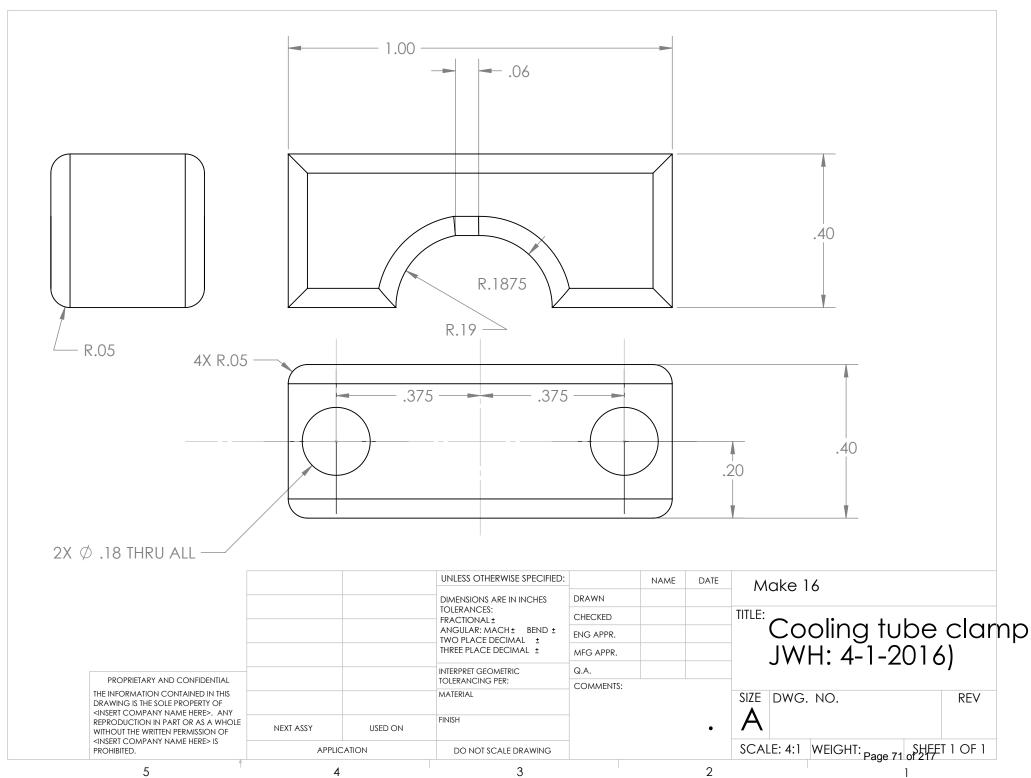


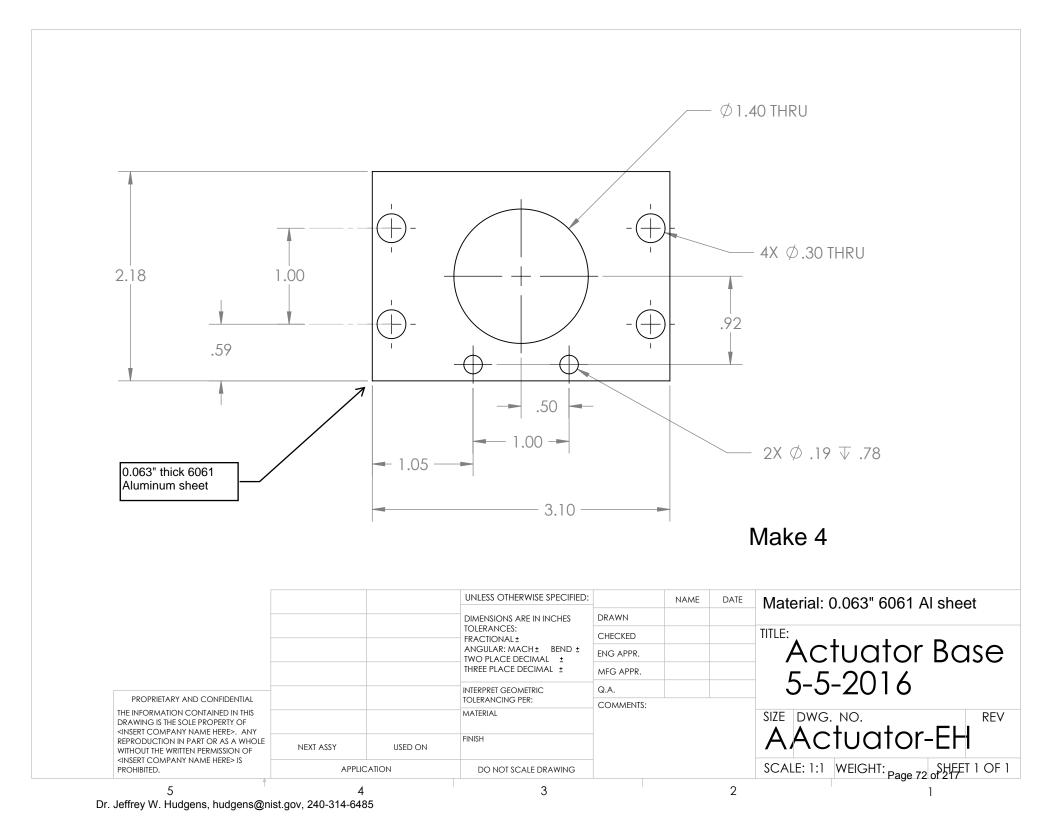


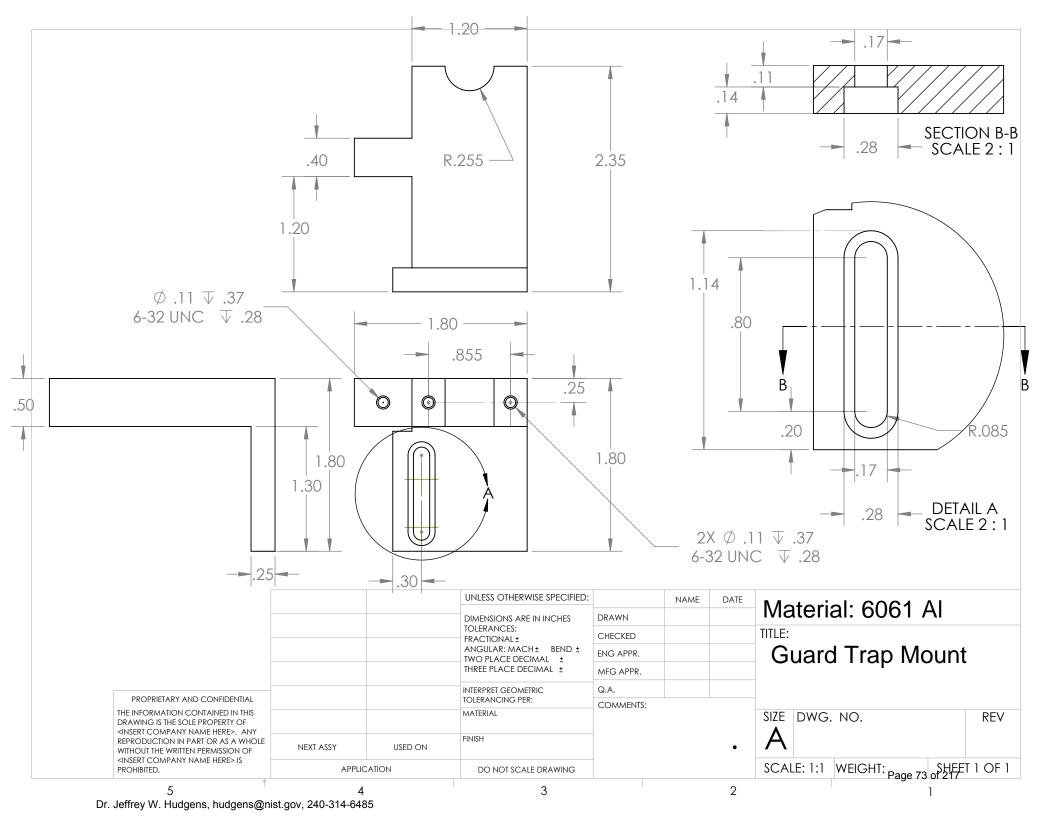


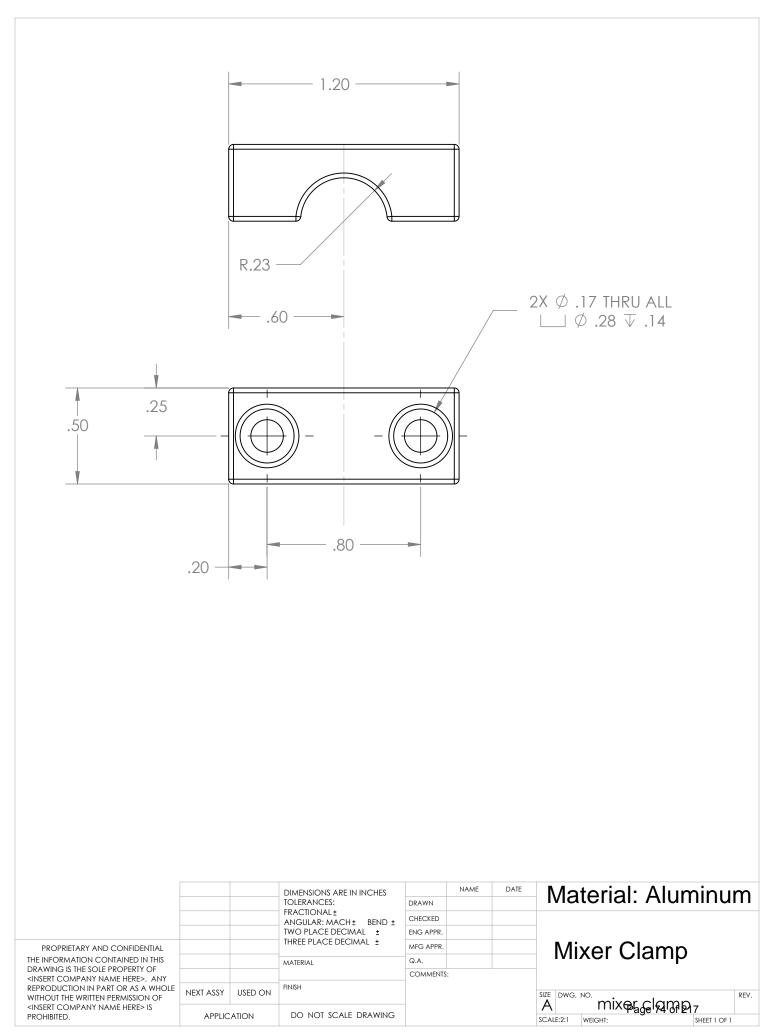




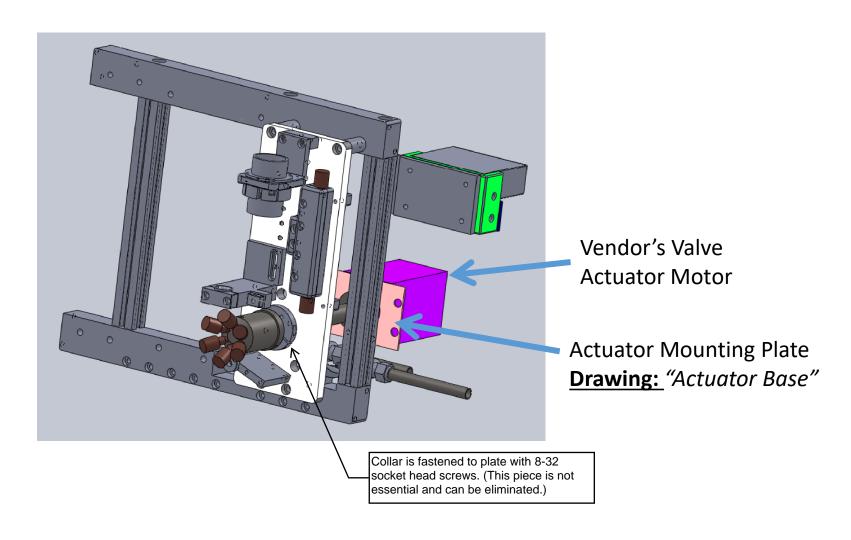




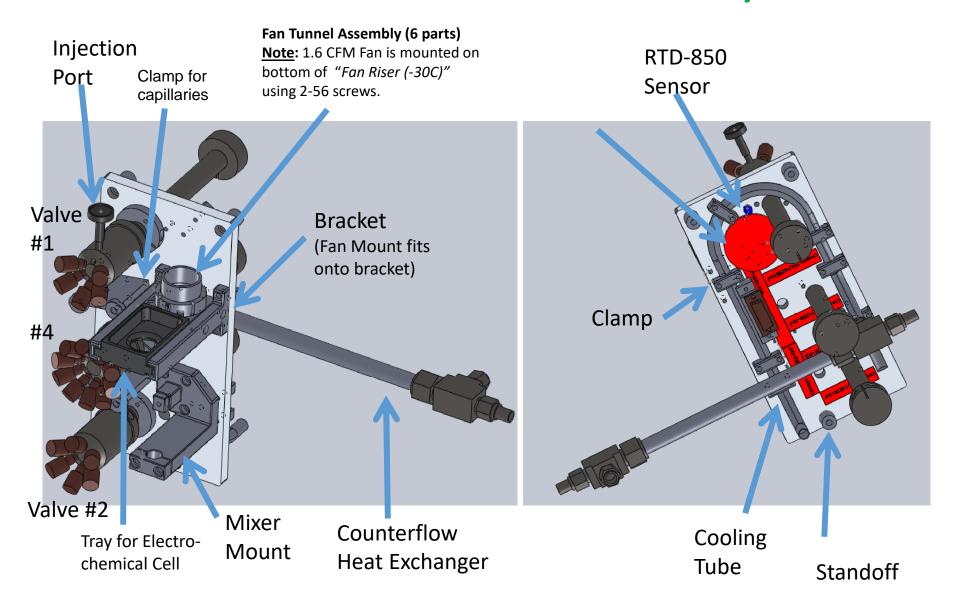


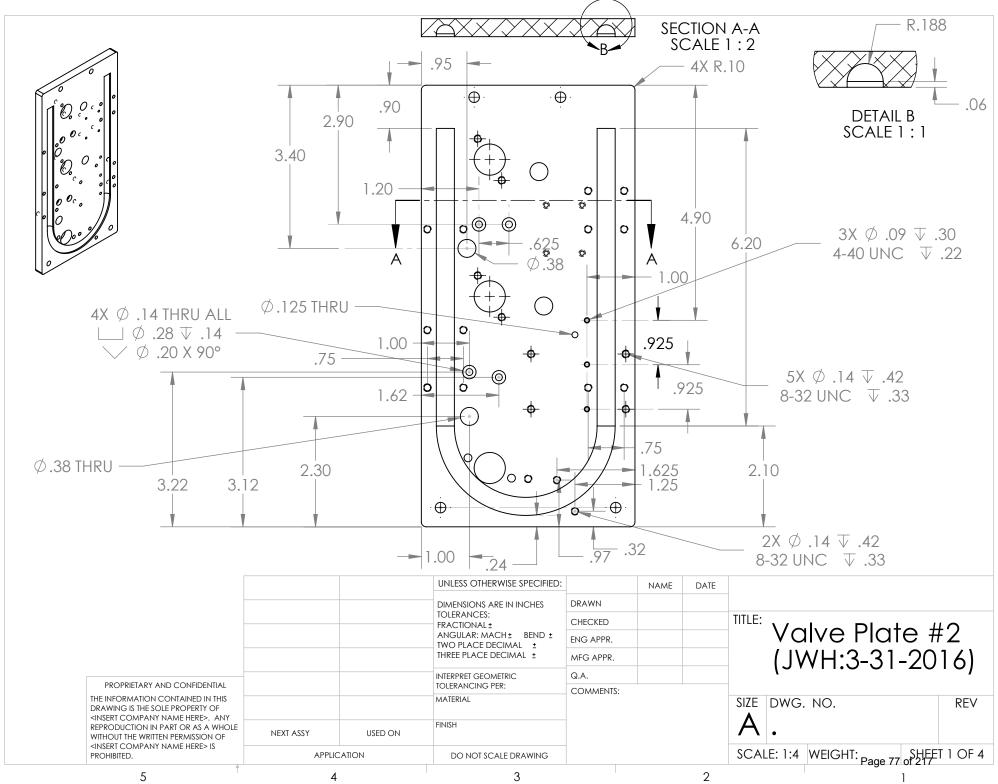


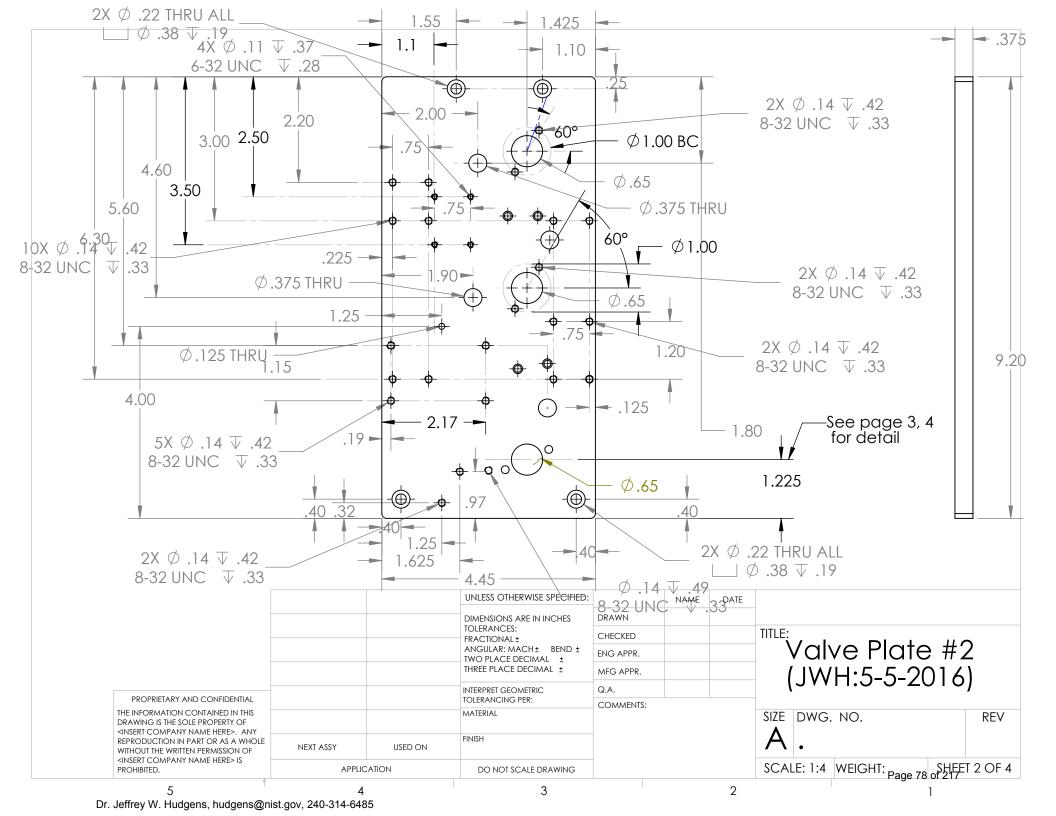
### Valve #3 Plate Mounted on Frame

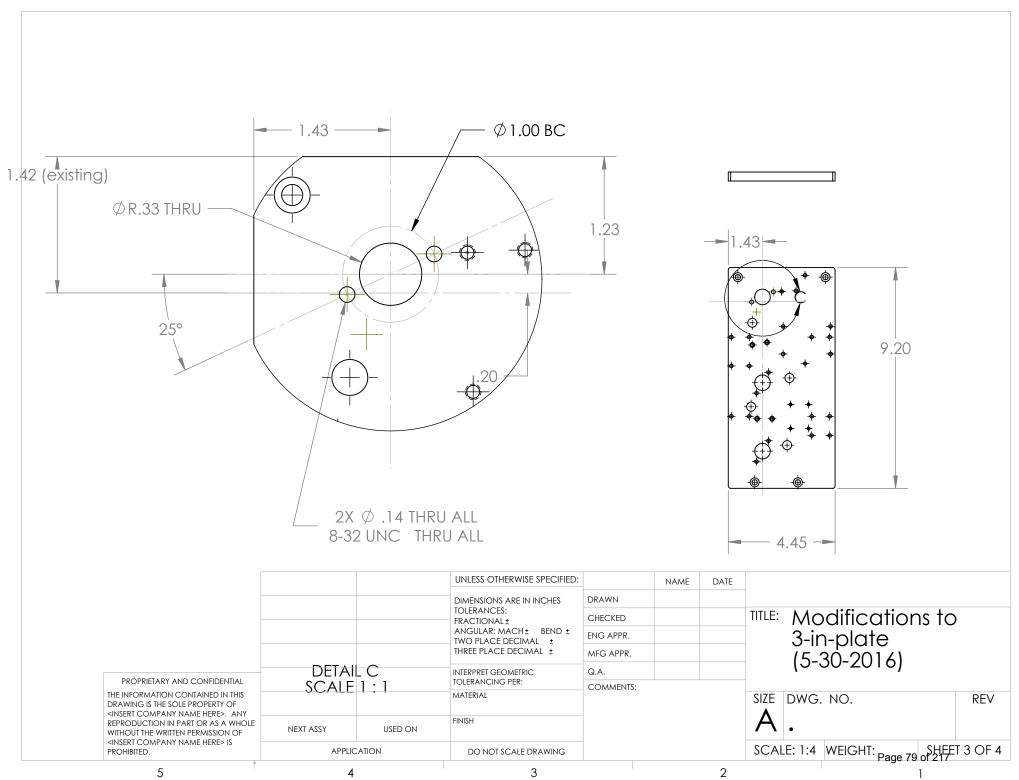


# 0 °C Chamber Plate Assembly

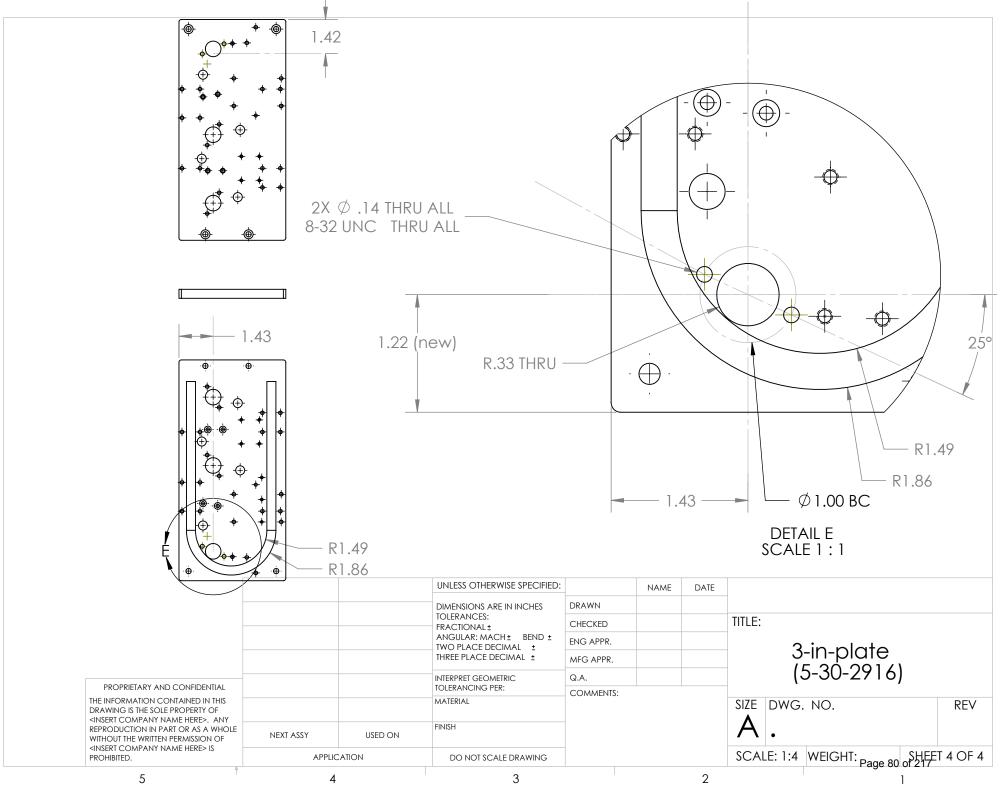


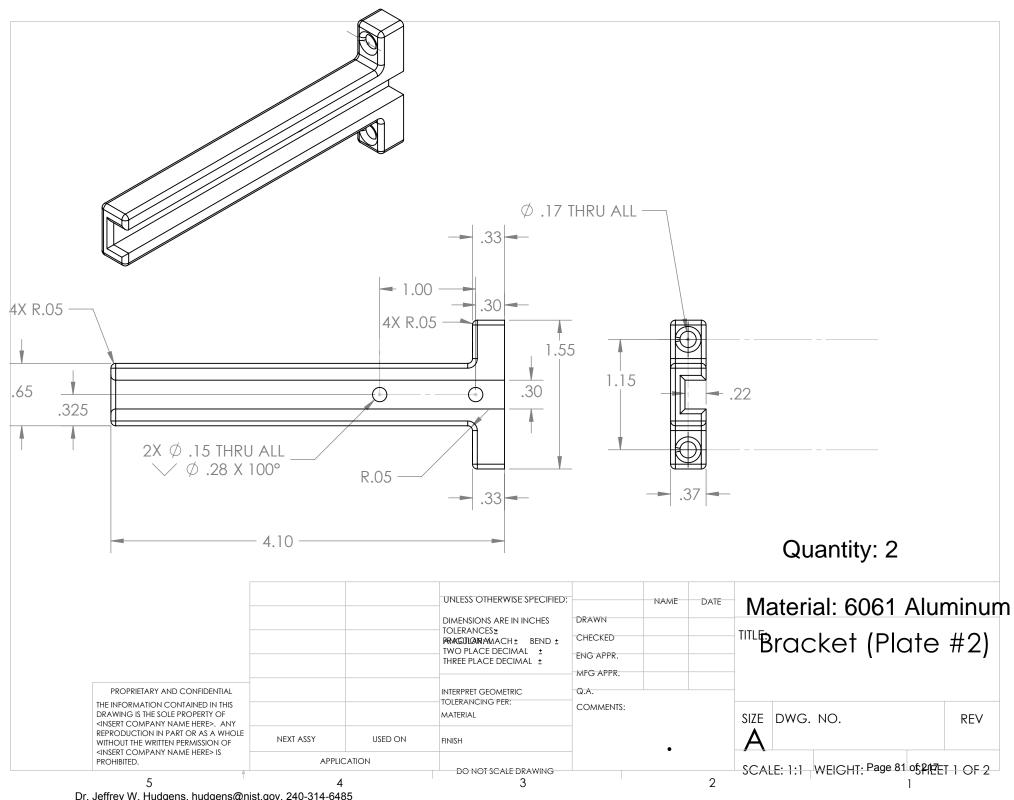


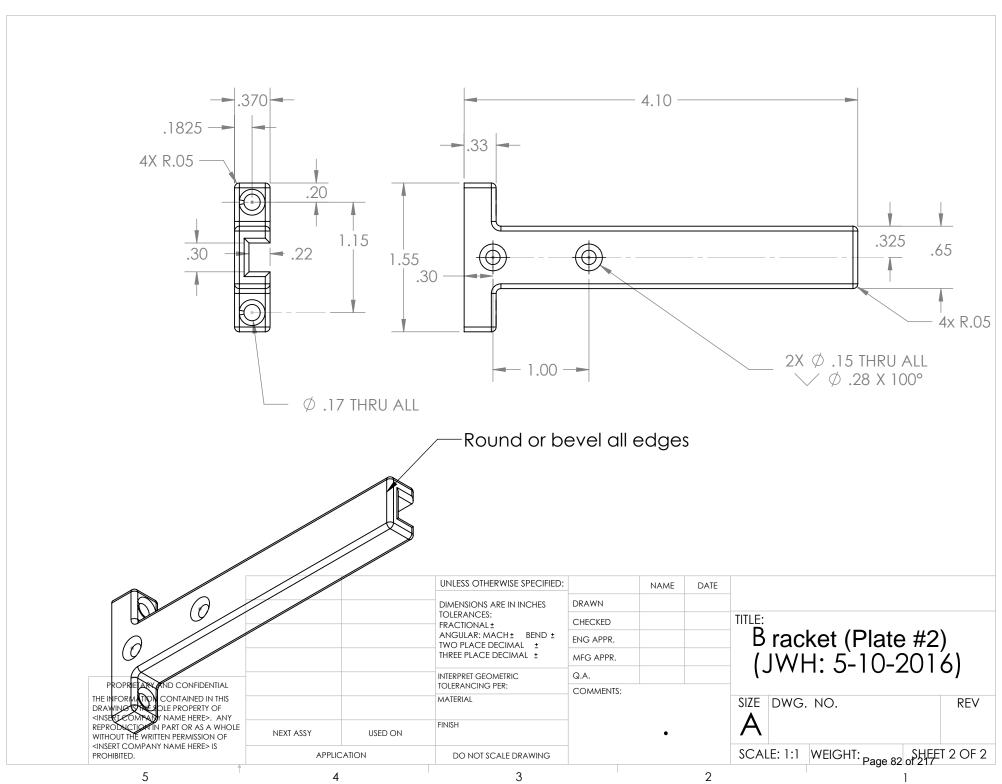




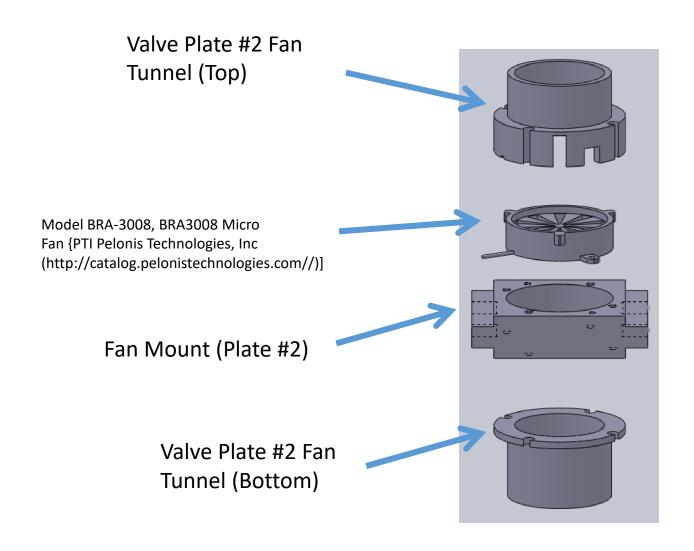
Dr. Jeffrey W. Hudgens, hudgens@nist.gov, 240-314-6485

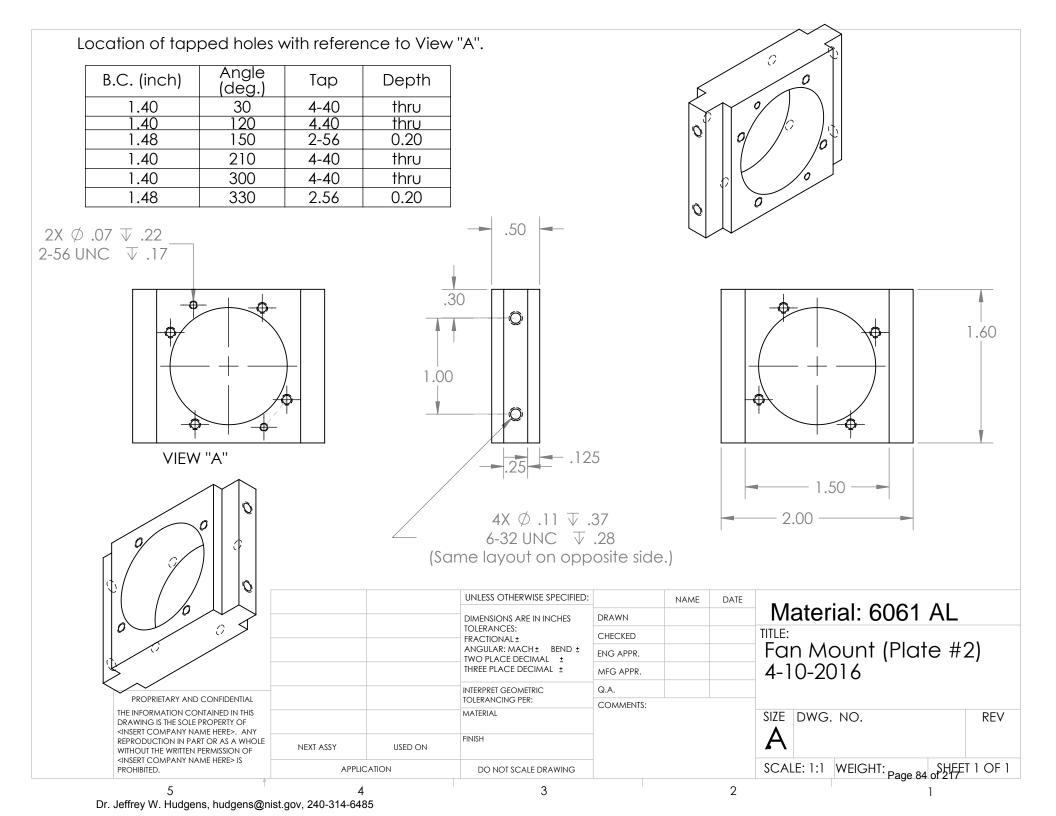


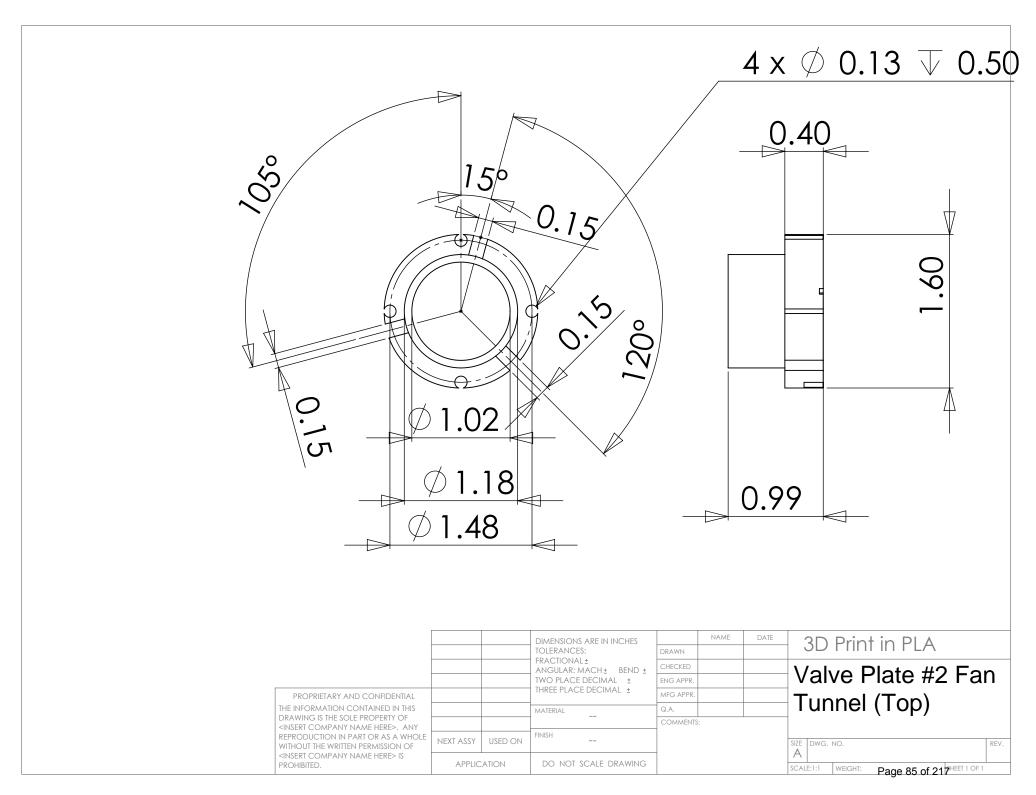


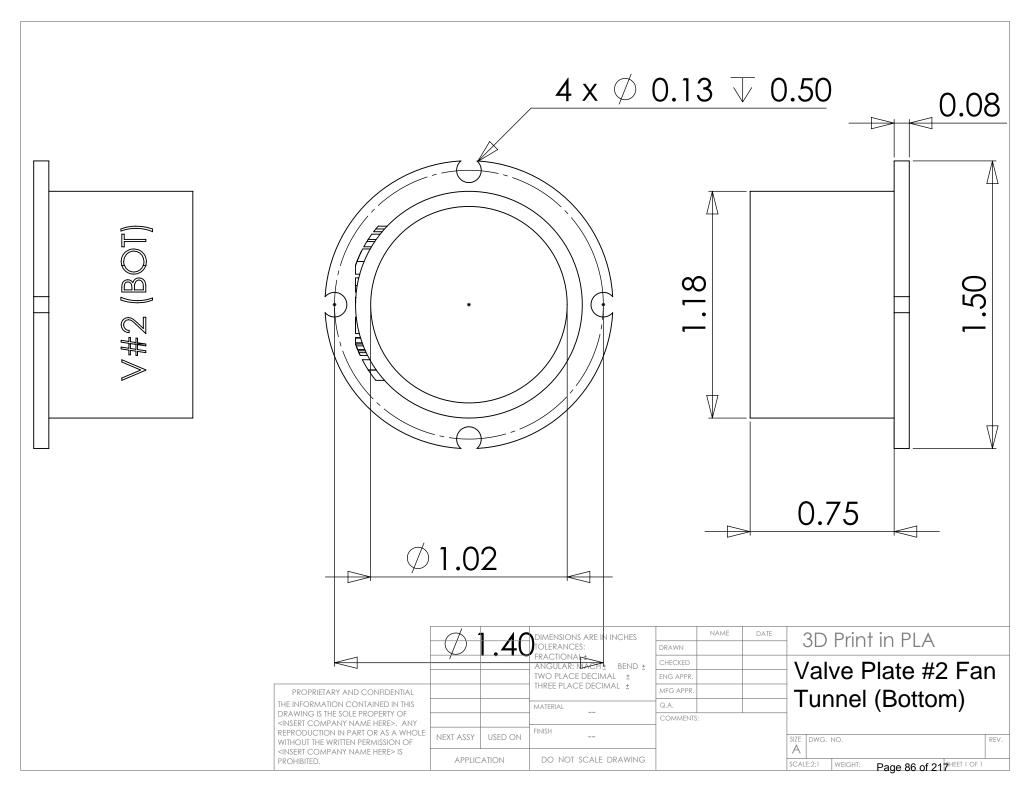


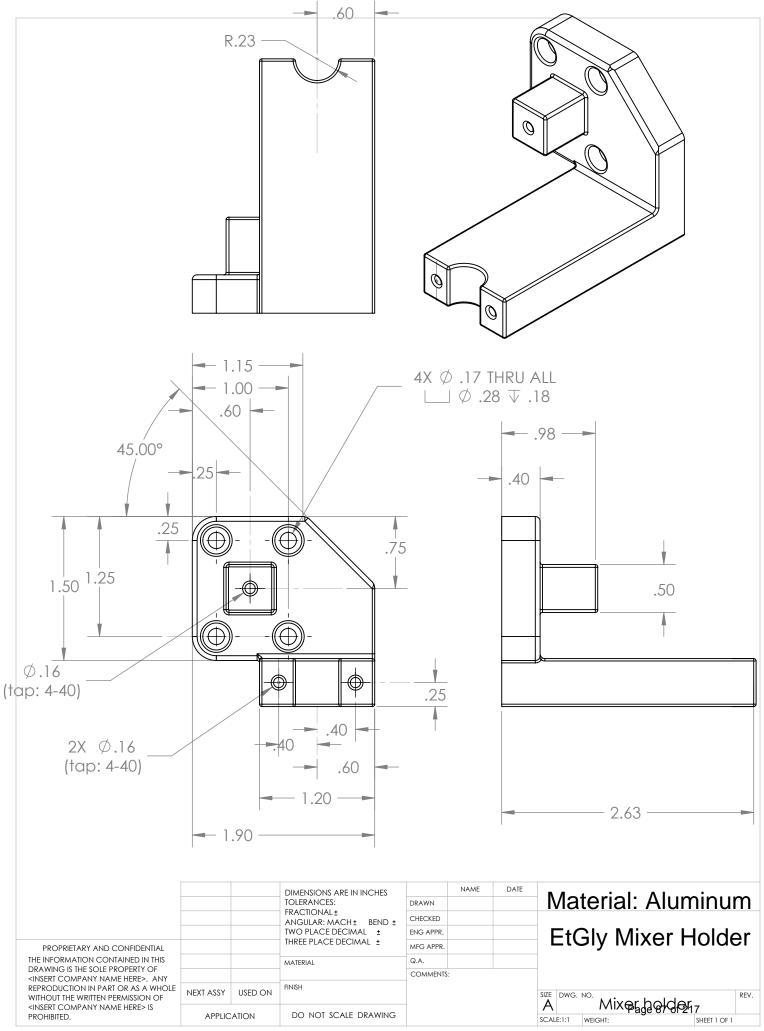
### Plate #2 Fan Assembly



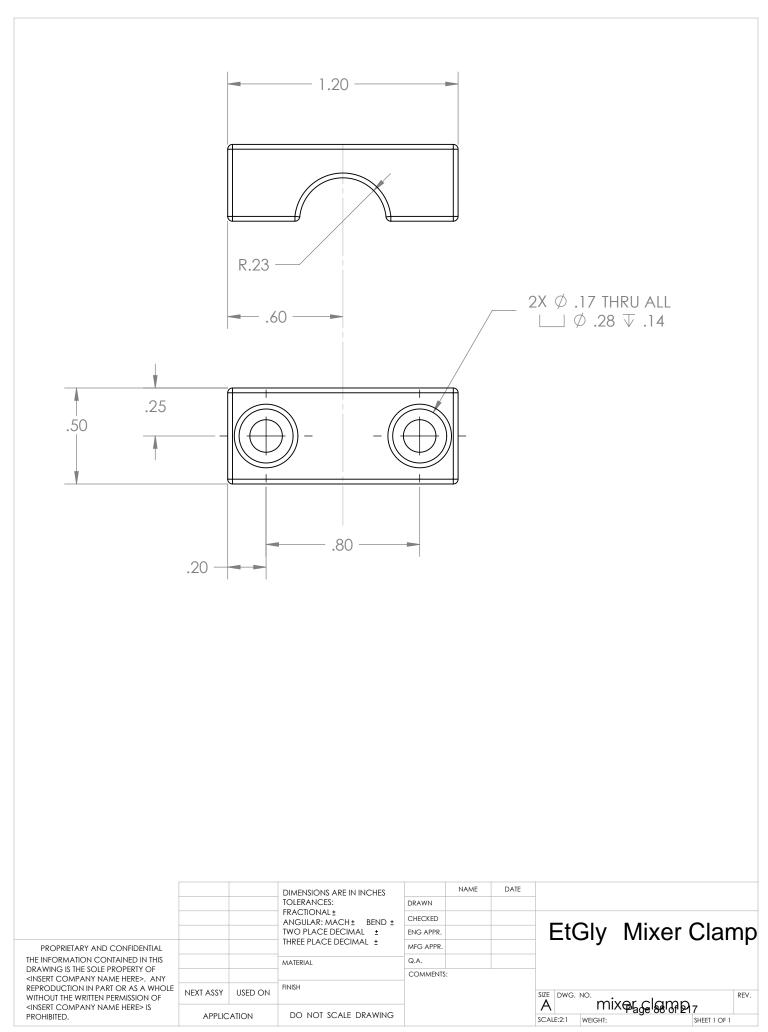


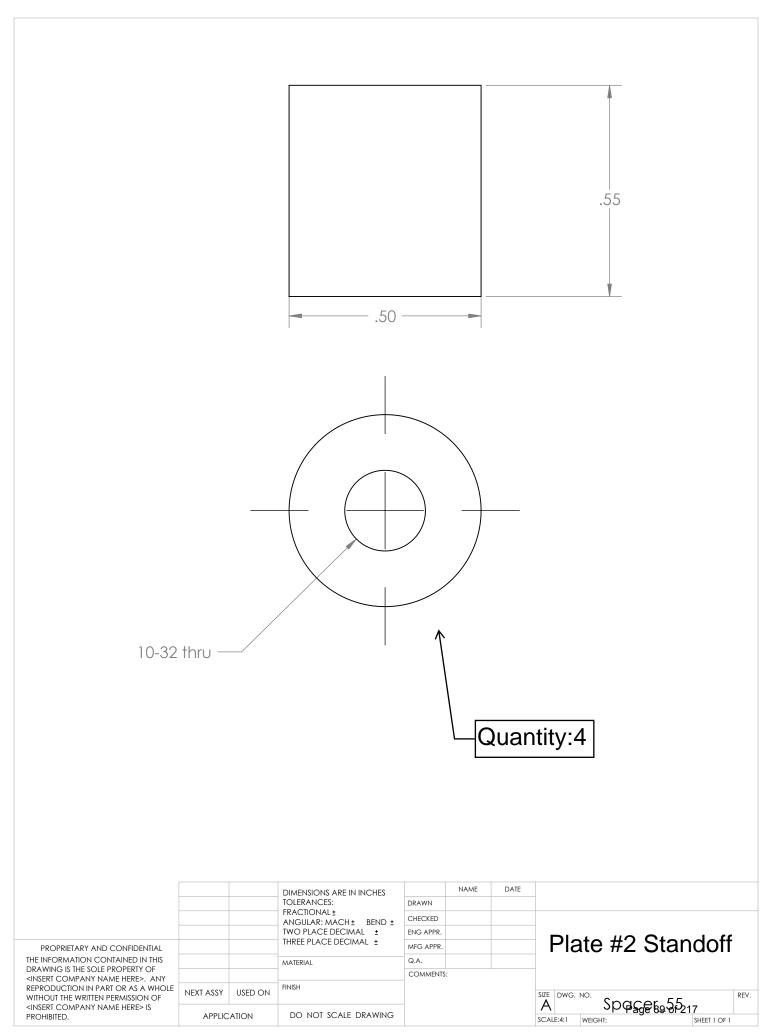


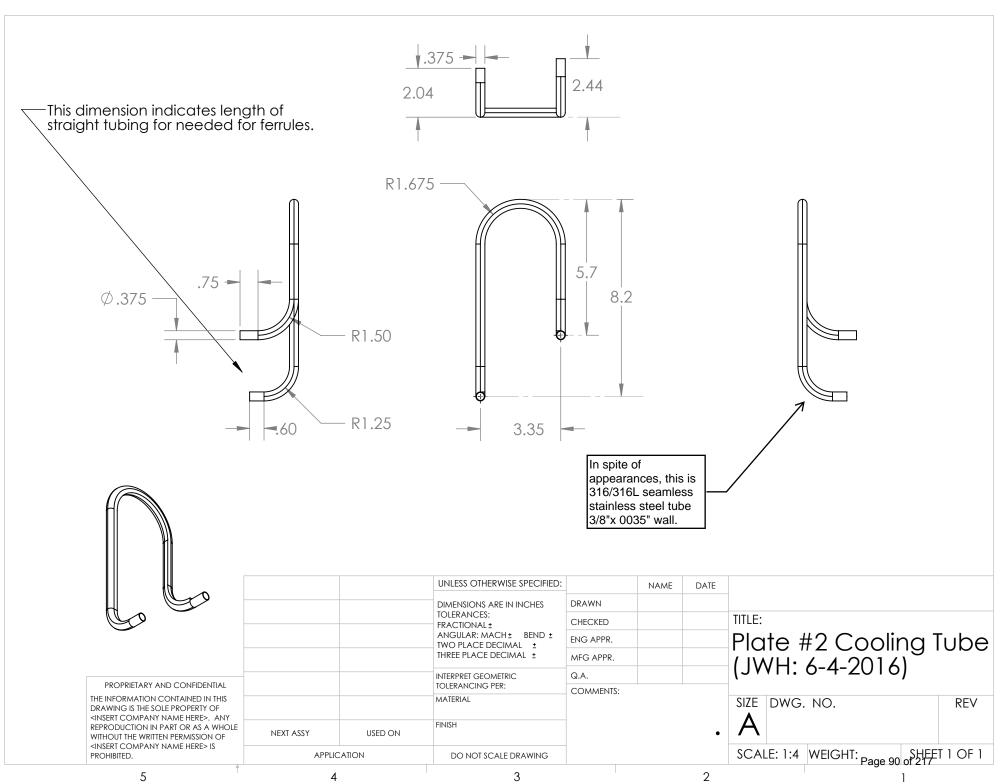


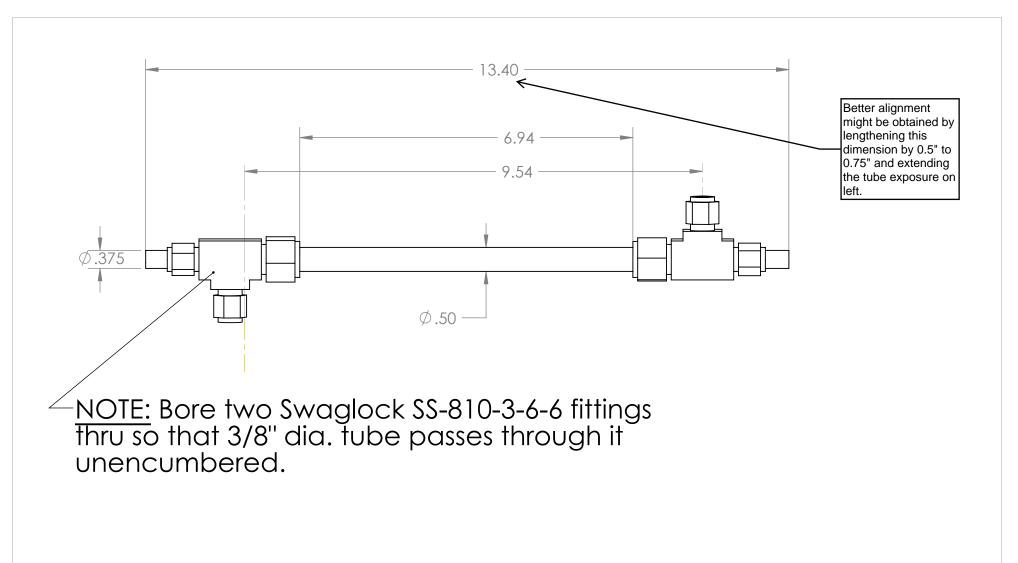


Dr. Jeffrey W. Hudgens, hudgens@nist.gov, 240-314-6485

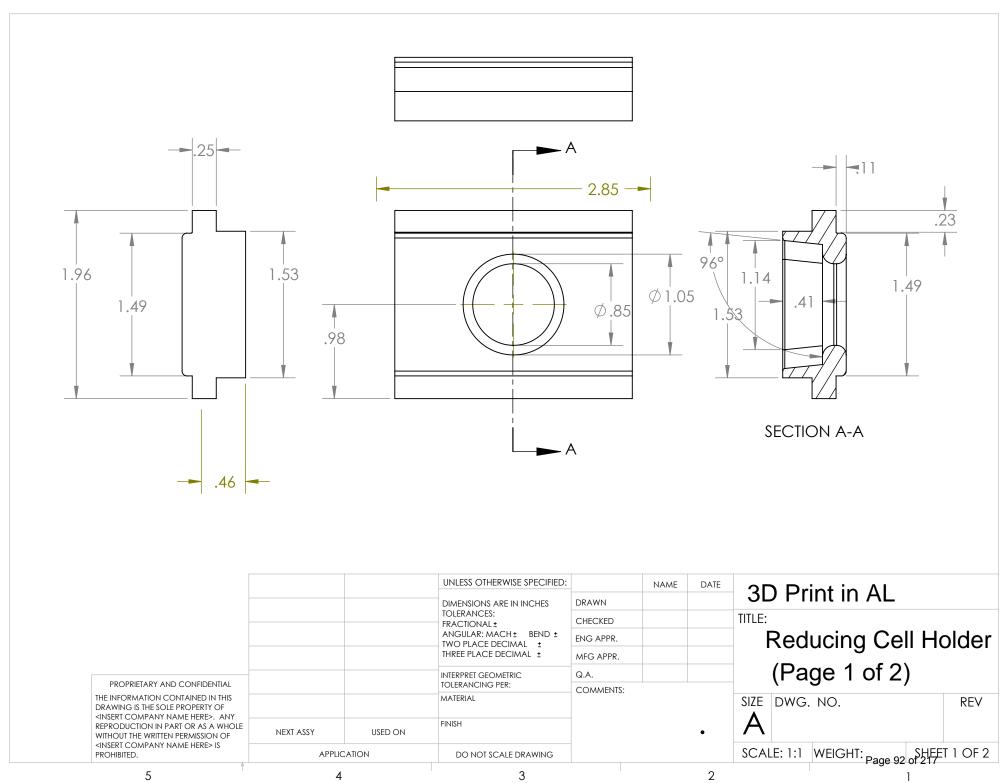


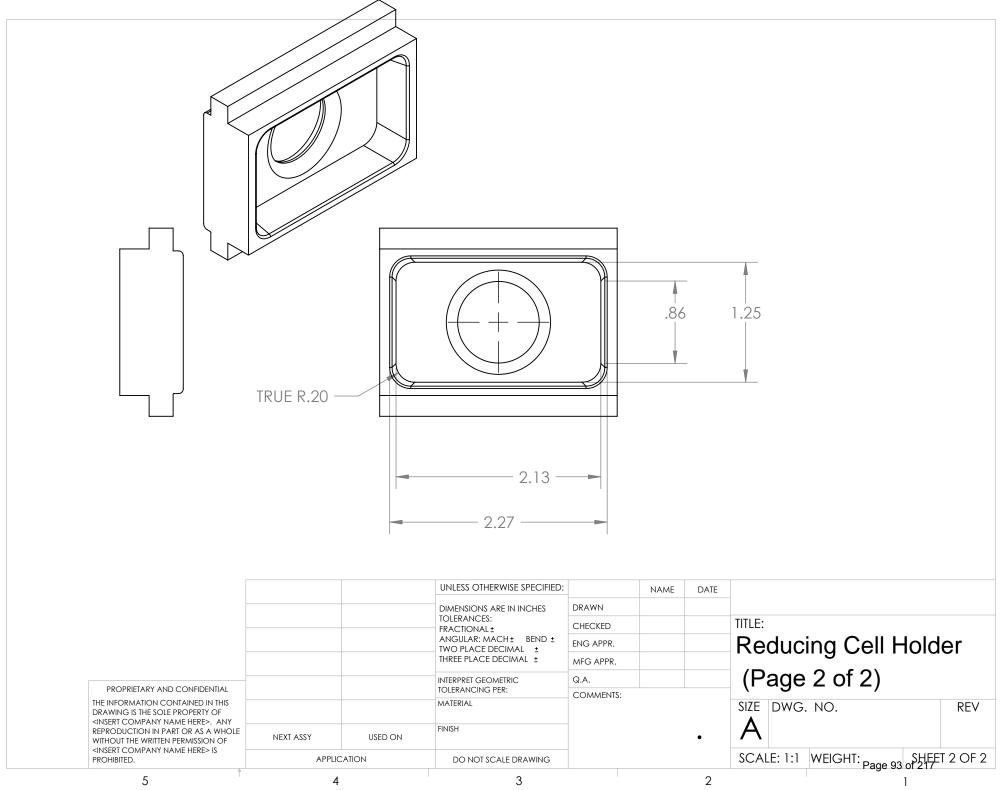






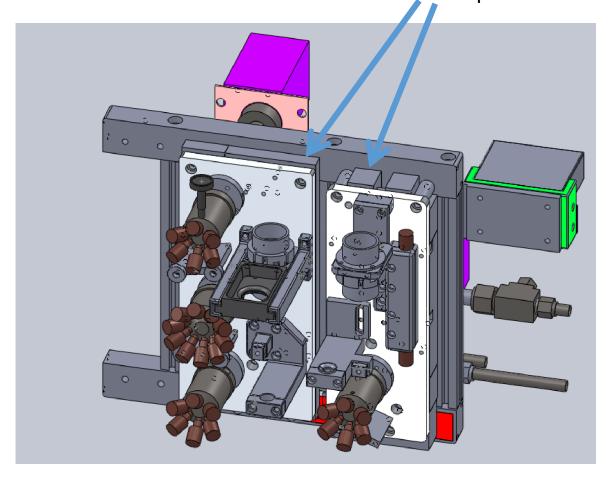
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PROPRIETARY AND CONFIDENTIAL THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF <insert a="" any="" as="" company="" heres.="" in="" name="" of<="" or="" part="" permission="" reproduction="" td="" the="" whole="" without="" written=""><td></td><td></td><td>DIMENSIONS ARE IN INCHES</td><td>DRAWN</td><td></td><td></td><td></td><td></td><td></td><td></td></insert>			DIMENSIONS ARE IN INCHES	DRAWN						
			TOLERANCES: FRACTIONAL ±	CHECKED			TITLE:	TLE: Heat Exchanger (JWH: 4-24-2016)		
			ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ± INTERPRET GEOMETRIC	ENG APPR.						
				MFG APPR.						
				Q.A.				(JVVII. 4-24-2010)		
			TOLERANCING PER:	COMMENTS:						
			MATERIAL				SIZE	DWG.	NO.	REV
	NEXT ASSY	USED ON	FINISH	_		./				
<insert company="" here="" name=""> IS PROHIBITED.</insert>	APPLICATION		DO NOT SCALE DRAWING				SCA	LE: 1:4	WEIGHT: Page 91	SHEET 1 OF 1
5	1		3			2			J	1



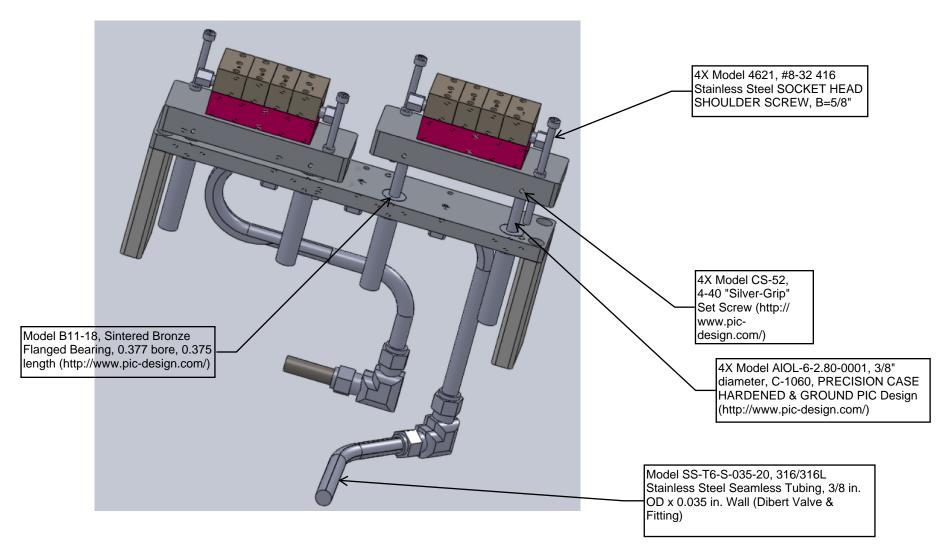


# Plates 2 & 3 on Frame

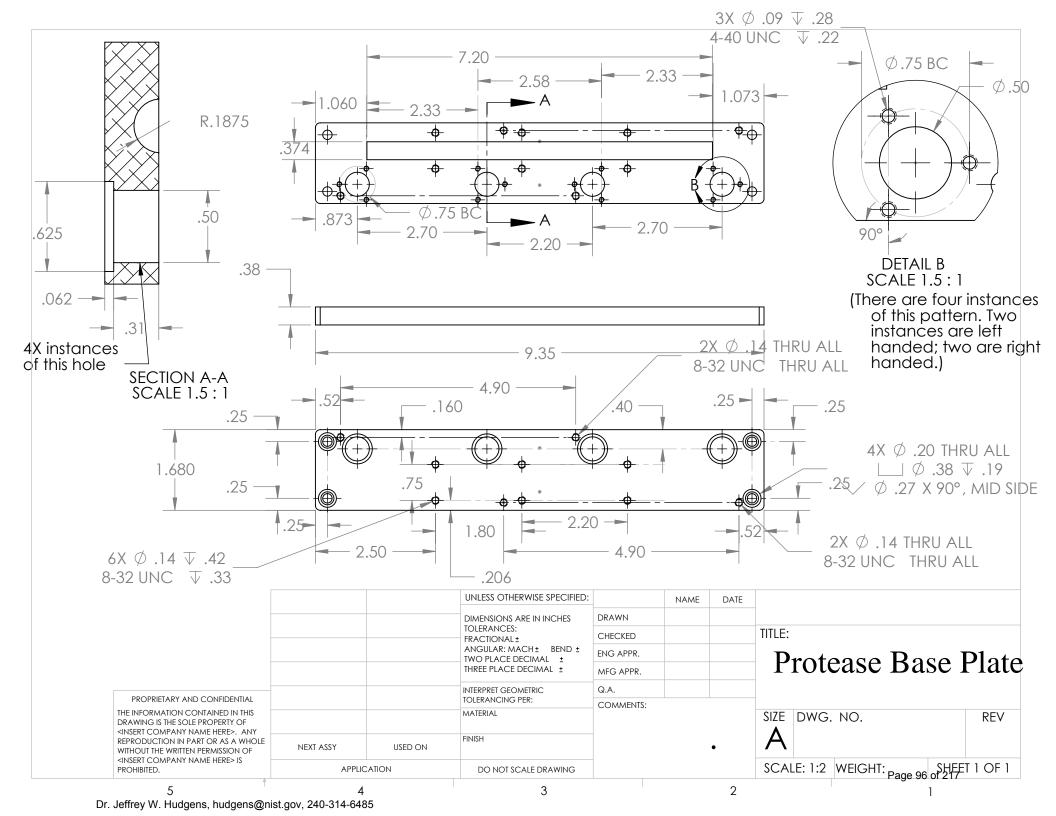
Foam Insulation covers back of these plates

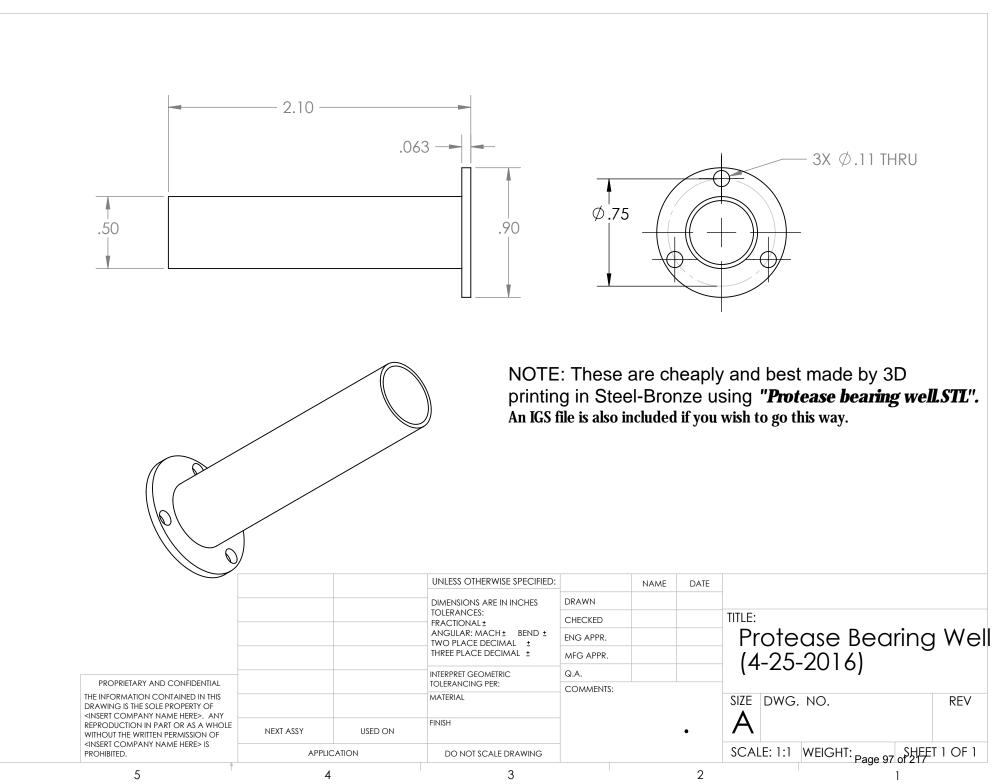


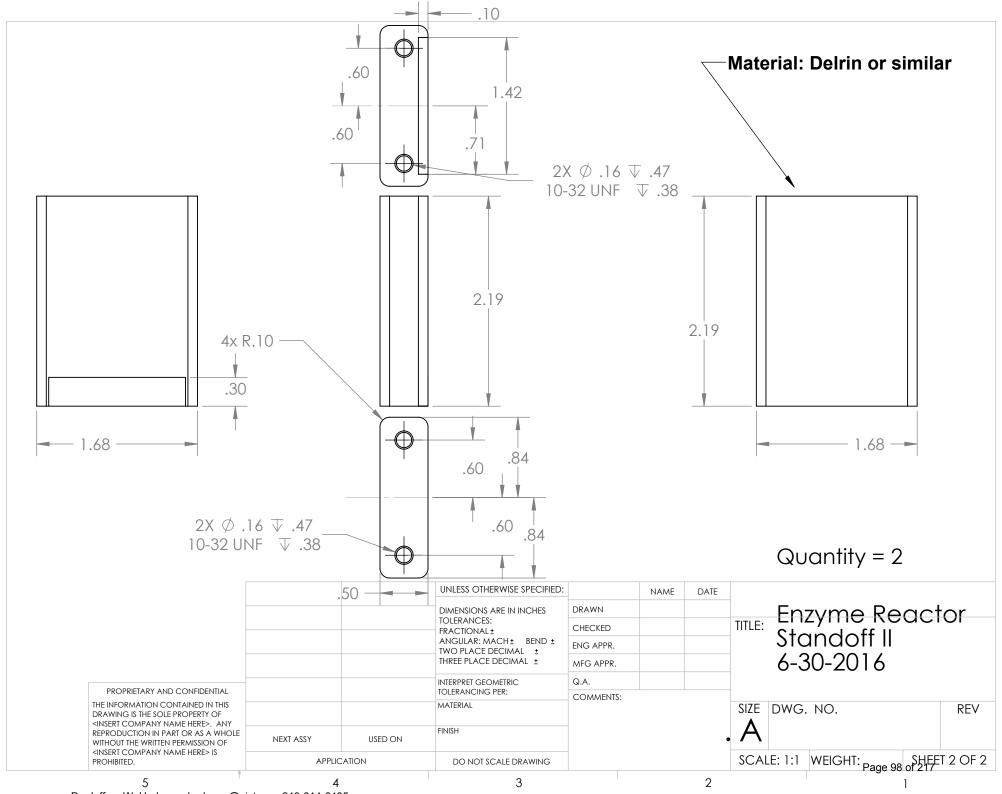
#### **Commercial Parts Incorporated into Protease Stage (Plate #1)**

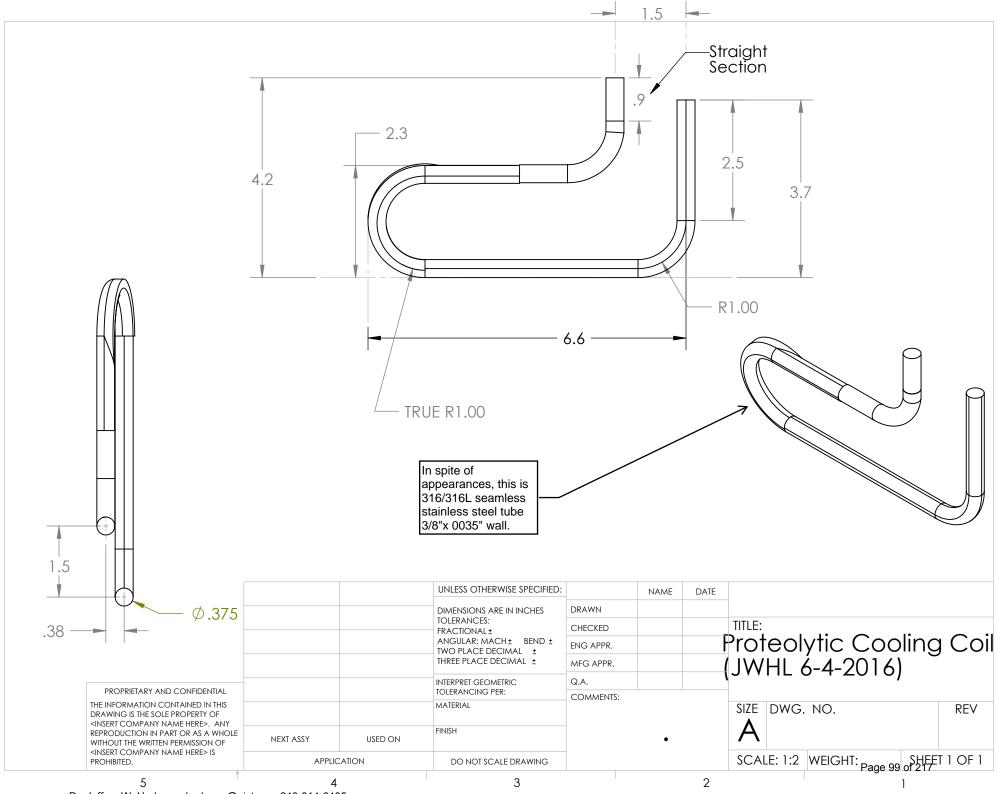


Protease columns on extendable mounts.

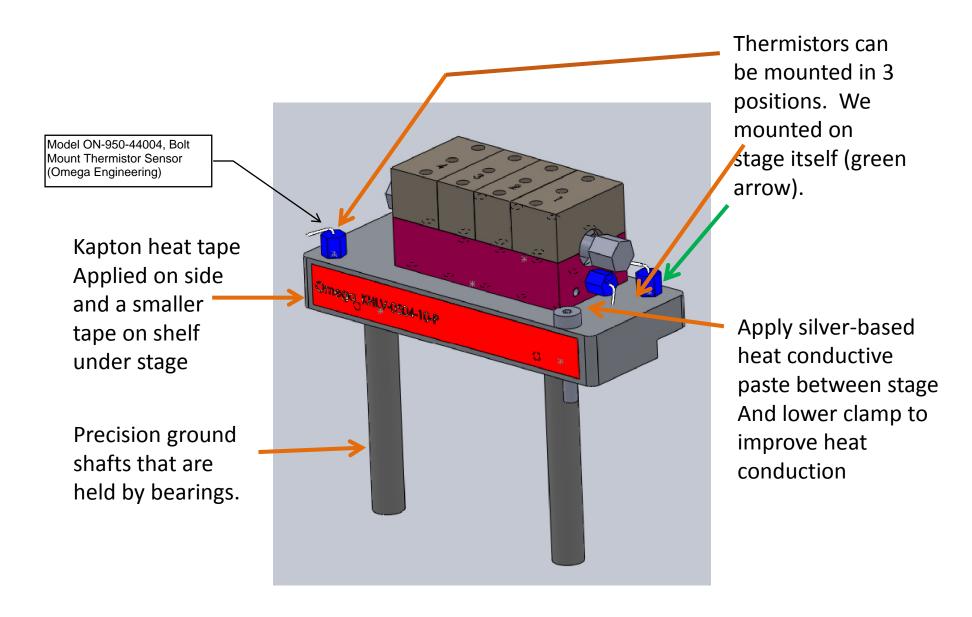


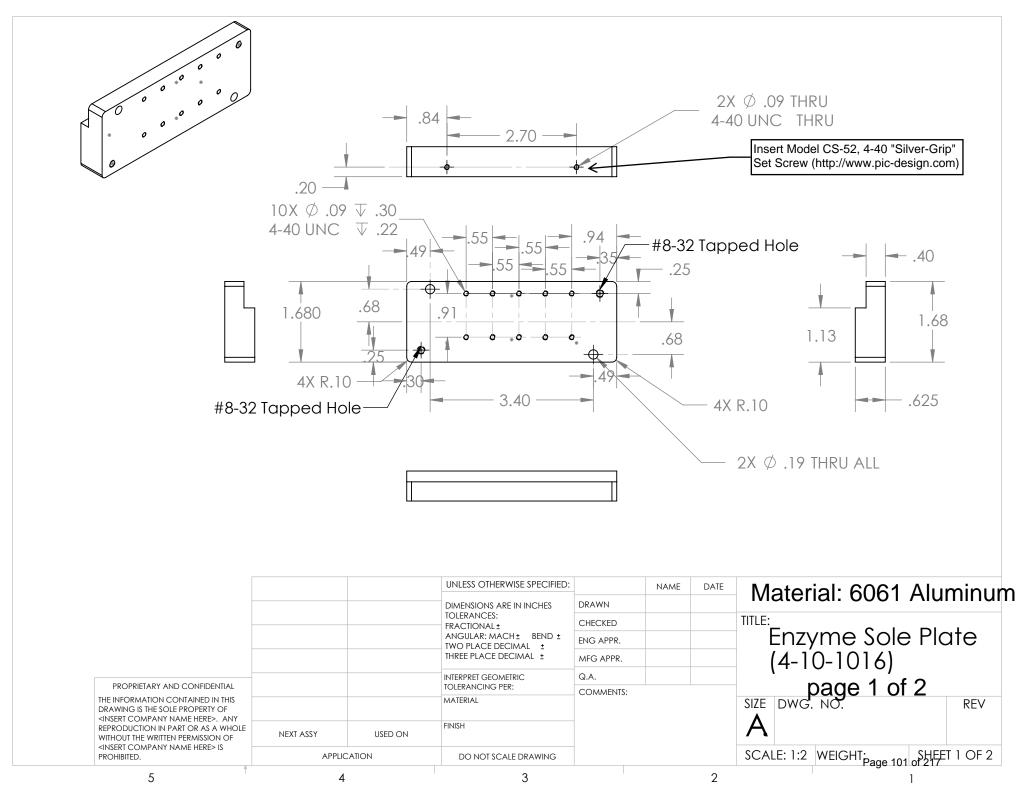


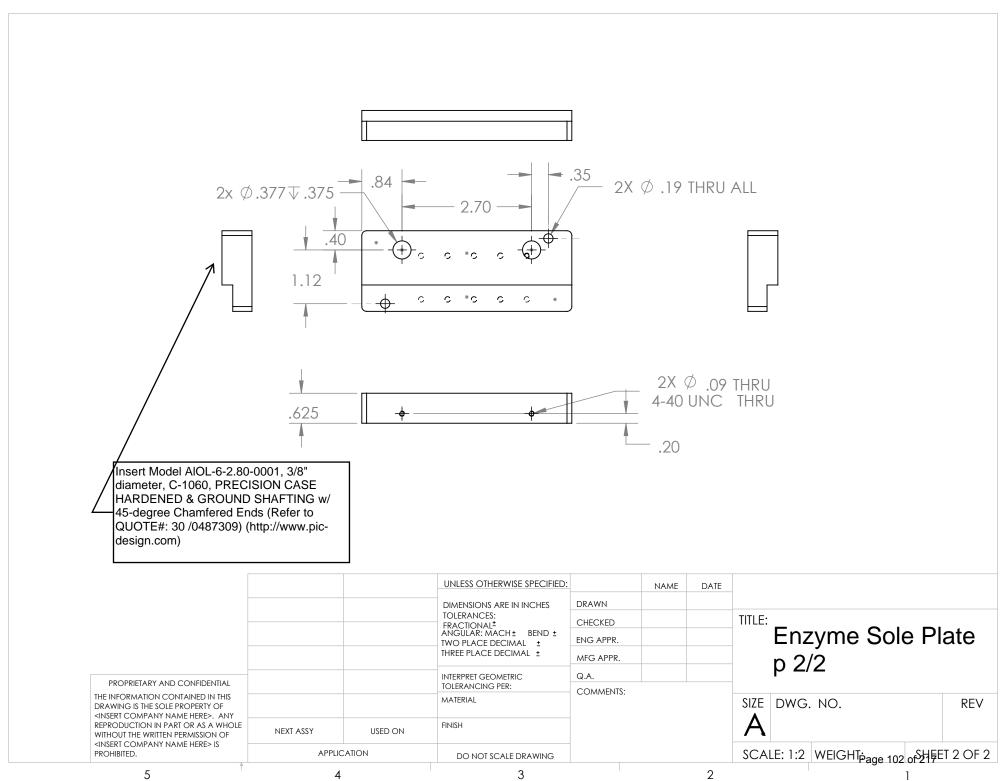


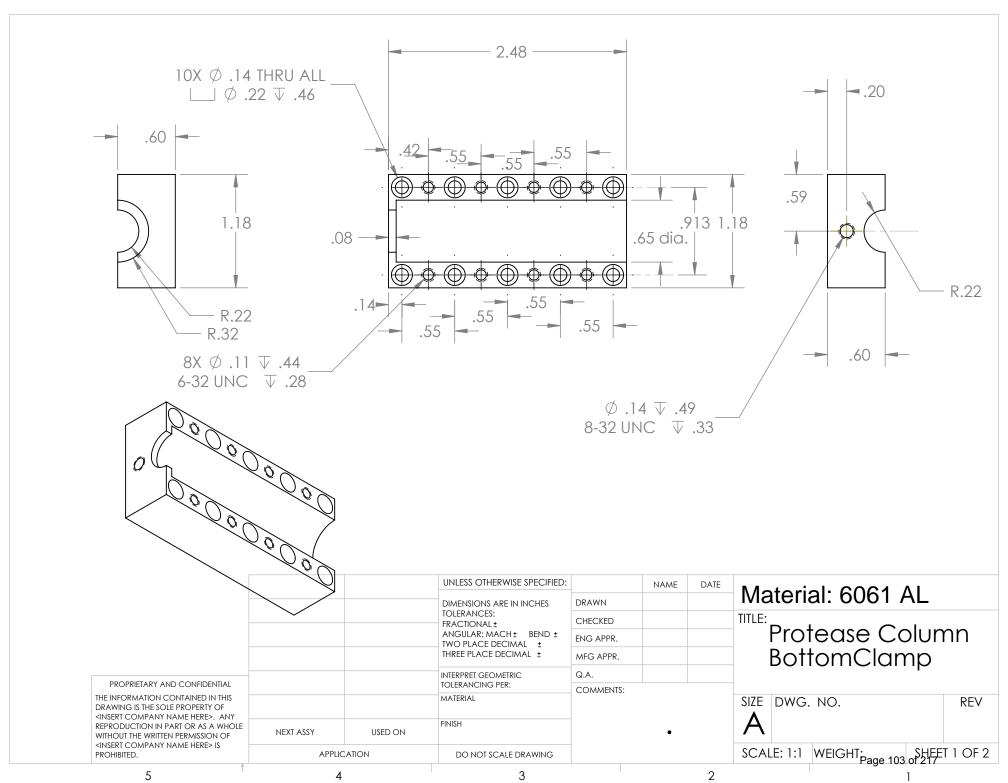


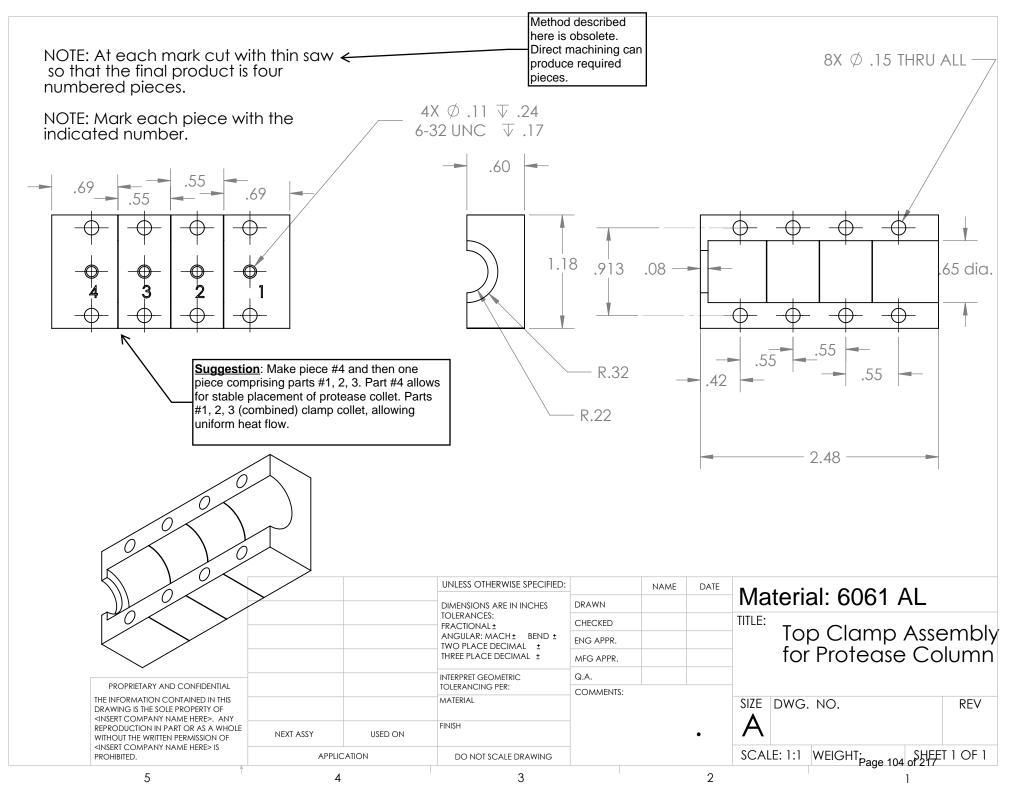
### Details of Protease Stage Assembly



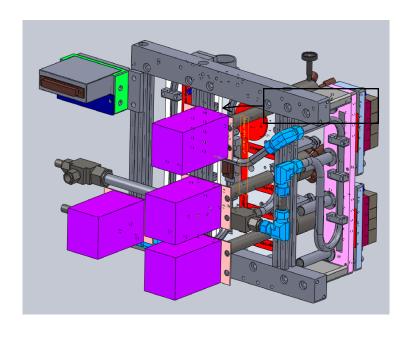


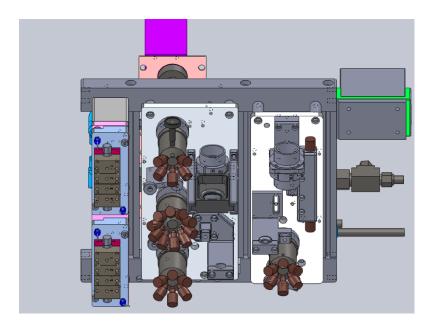




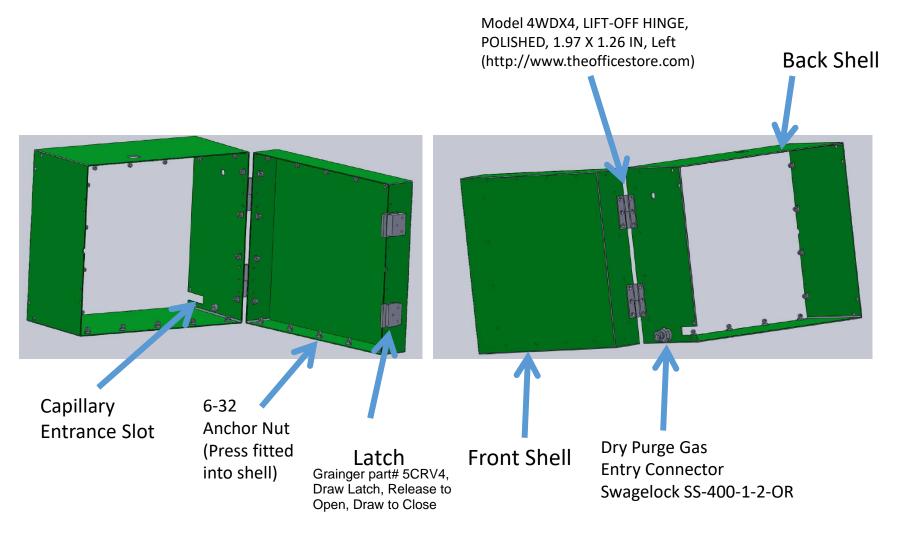


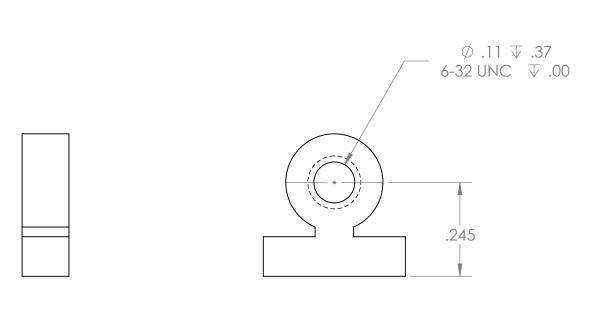
## Assembly View of Frame + 3 Plates





### Overview of Shell Enclosure

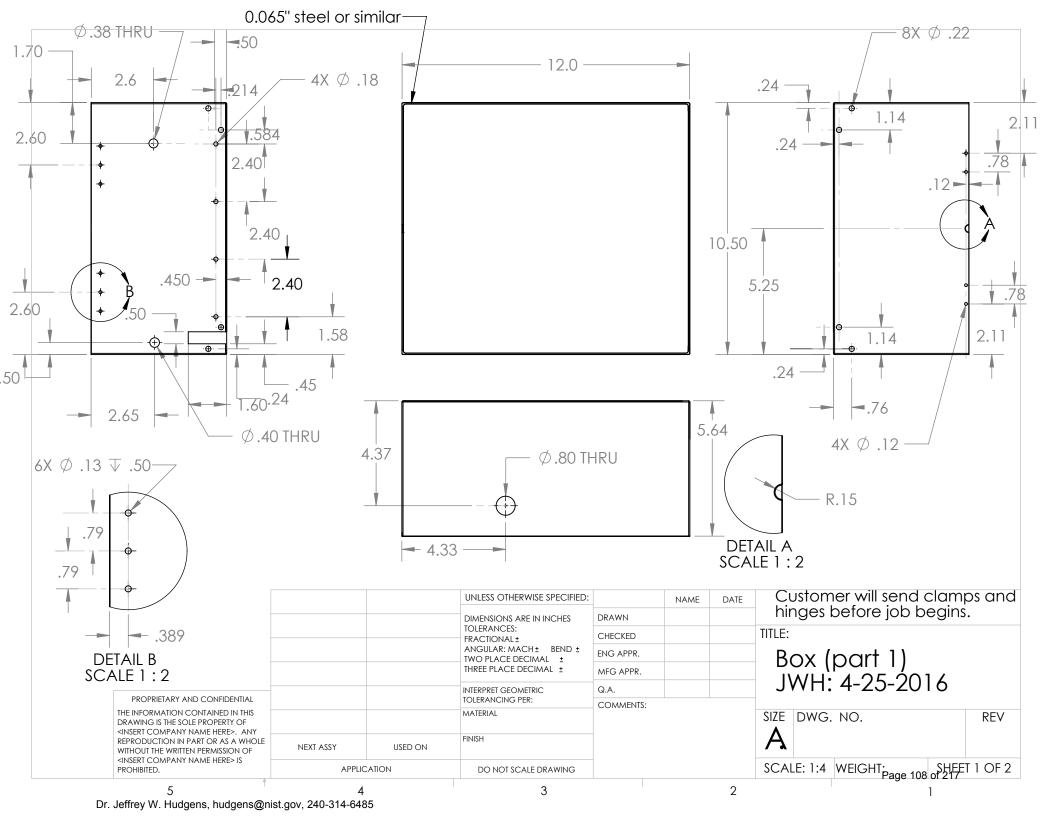


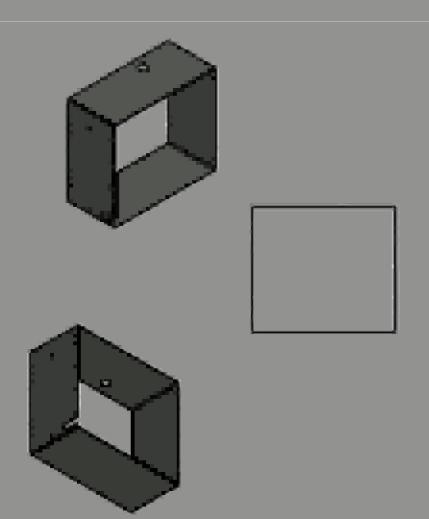


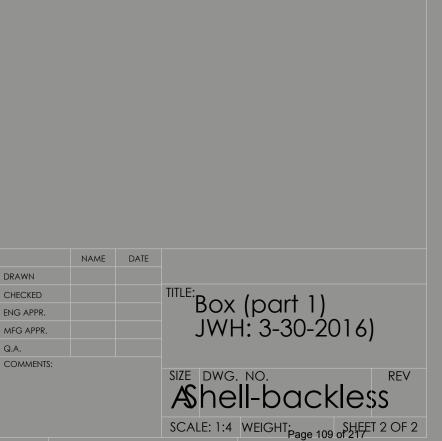
These anchor nuts are available comercially.

			UNLESS OTHERWISE SPECIFIED:		NAME	DATE			
			DIMENSIONS ARE IN INCHES	DRAWN					
			TOLERANCES: FRACTIONAL ±	CHECKED			TITLE:		
			ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±	ENG APPR.			A 1 DT /		
				MFG APPR.			Anchor Nut		
PROPRIETARY AND CONFIDENTIAL			INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.					
THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF SINSERT COMPANY NAME HERES, ANY			MATERIAL	COMMENTS:			SIZE DWG. NO. REV		
REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF	NEXT ASSY	USED ON	FINISH				A Anchor Nut		
<insert company="" here="" name=""> IS PROHIBITED.</insert>	APPLICATION		DO NOT SCALE DRAWING				SCALE: 4:1 WEIGHT Page 107 of 217		
5	4		3			2	1		

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NEXT ASSY USED ON APPLICATION DO NOT SCALE DRAWING

3

Q.A.

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES

ANGULAR: MACH ± BEND ±

TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC

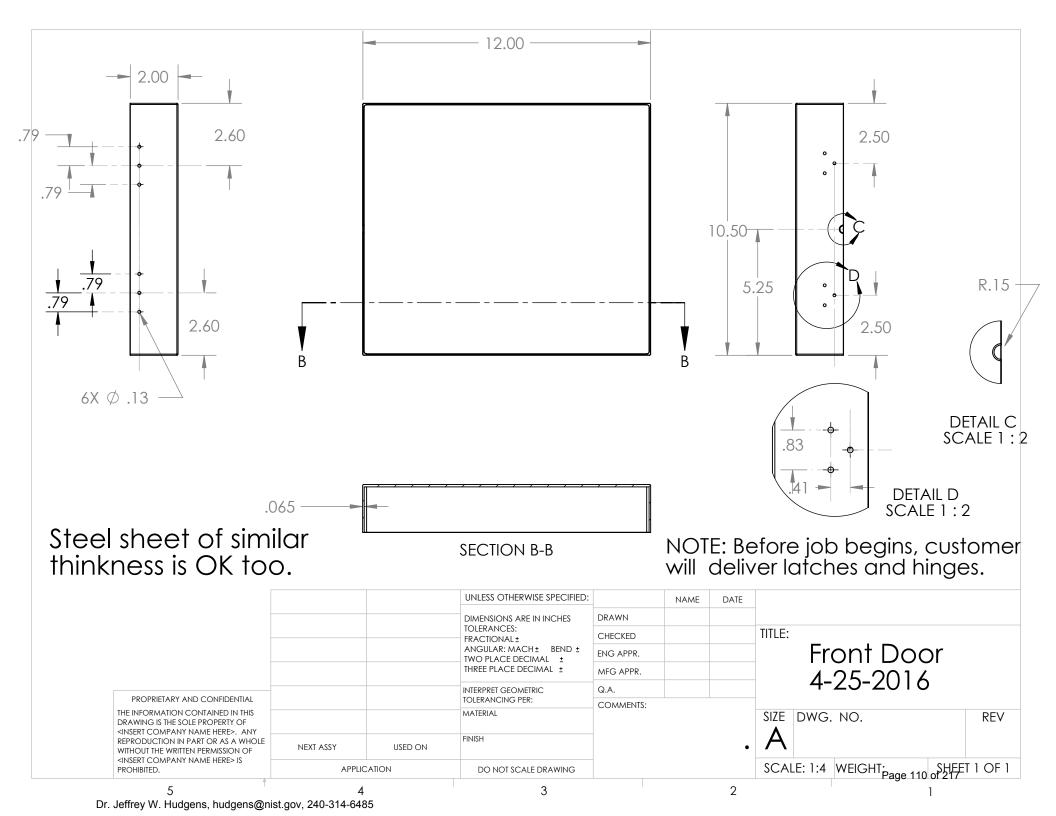
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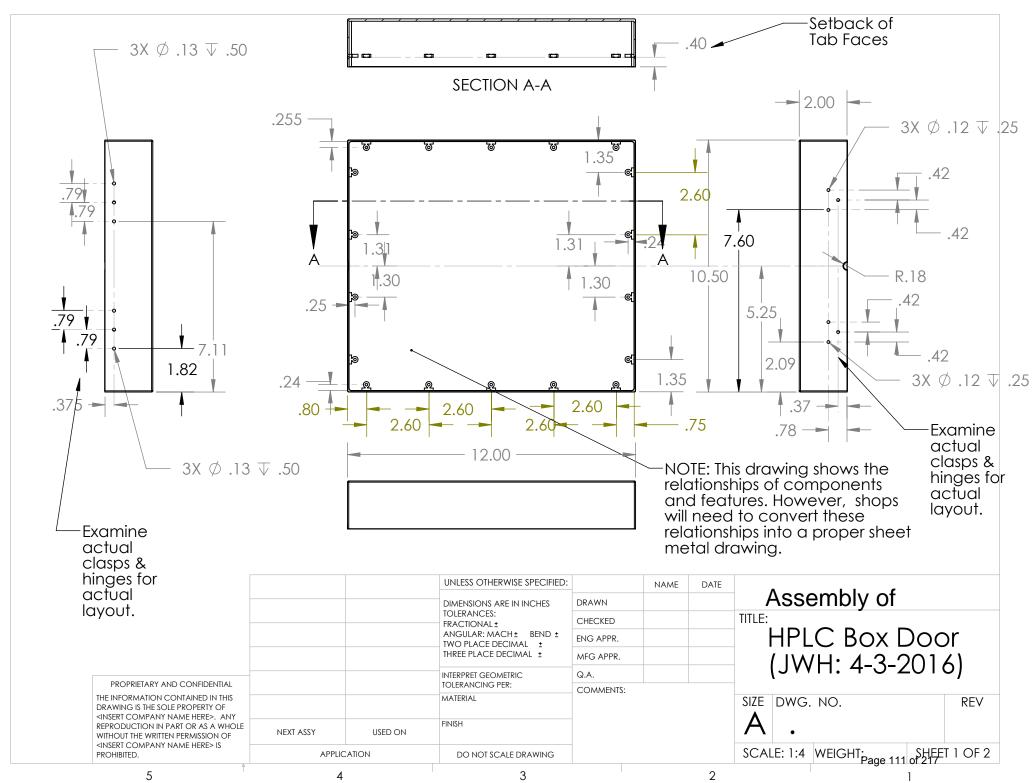
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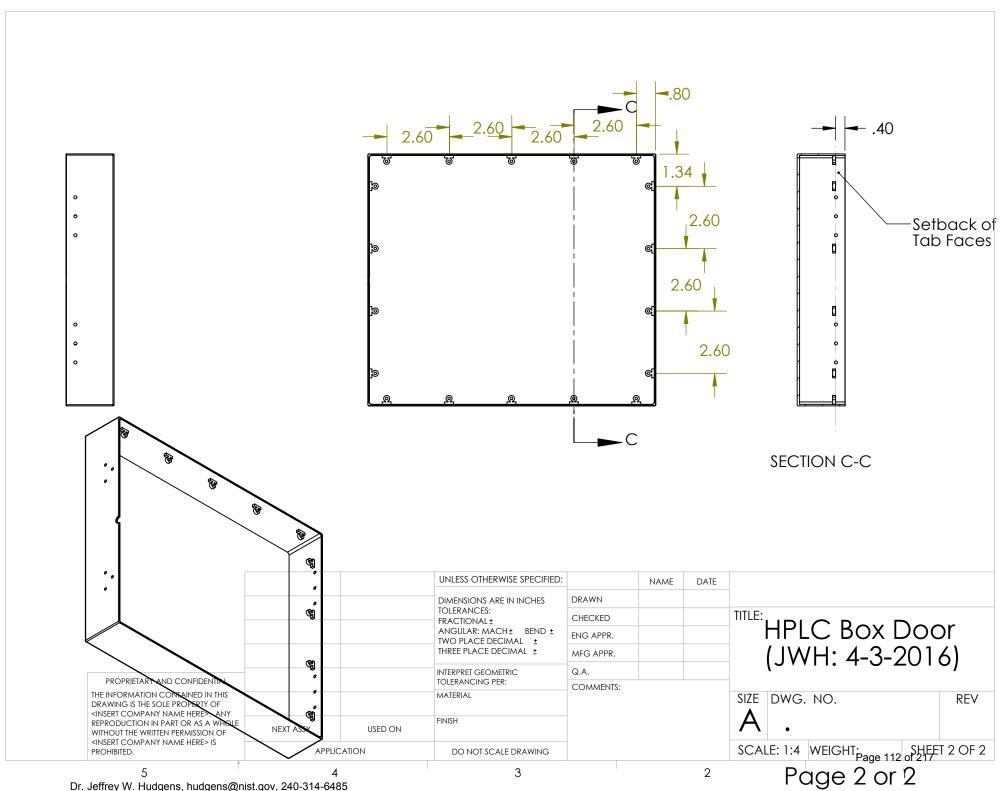
FINISH

TOLERANCES:

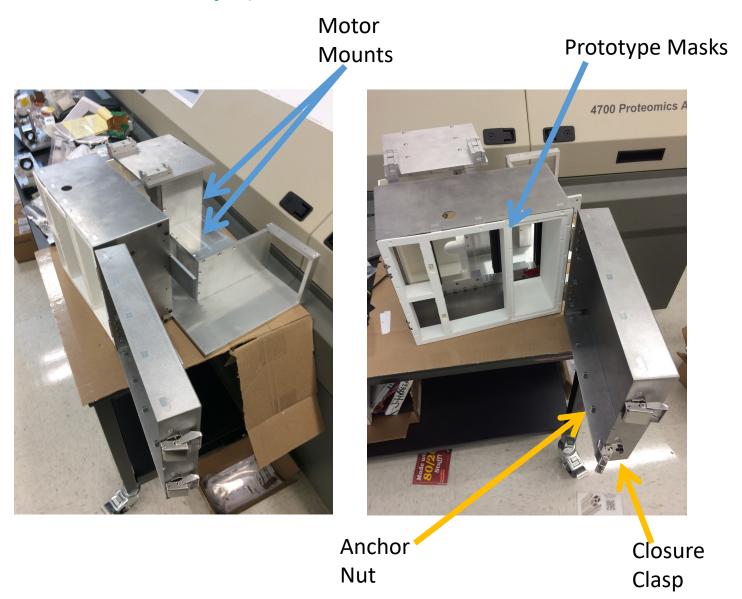
FRACTIONAL ±



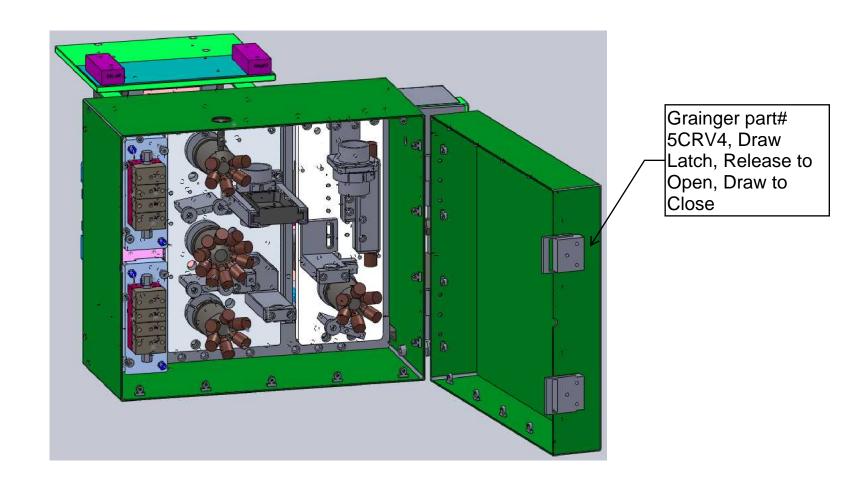




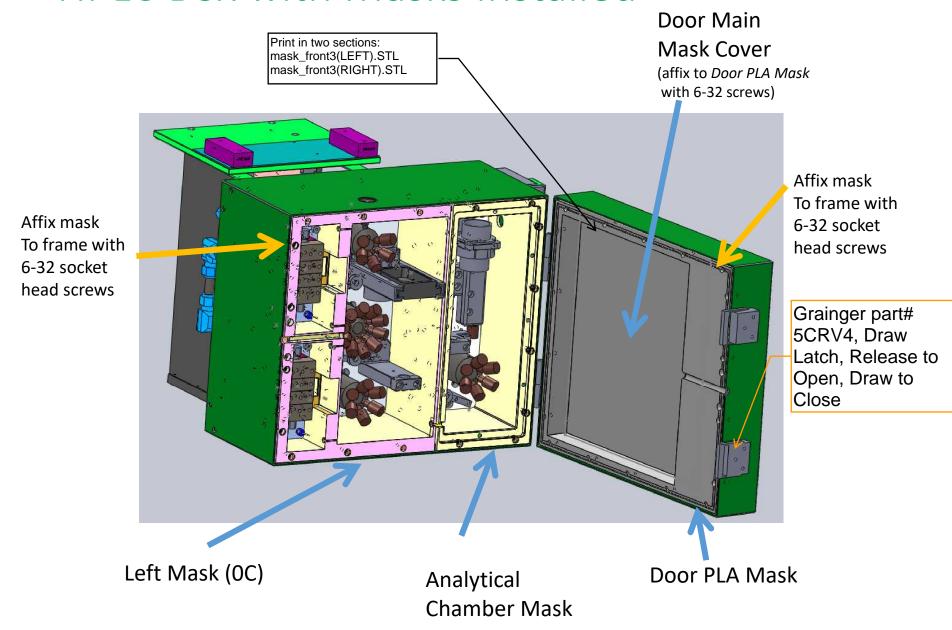
### Box Assembly (Before Surface Treatments)



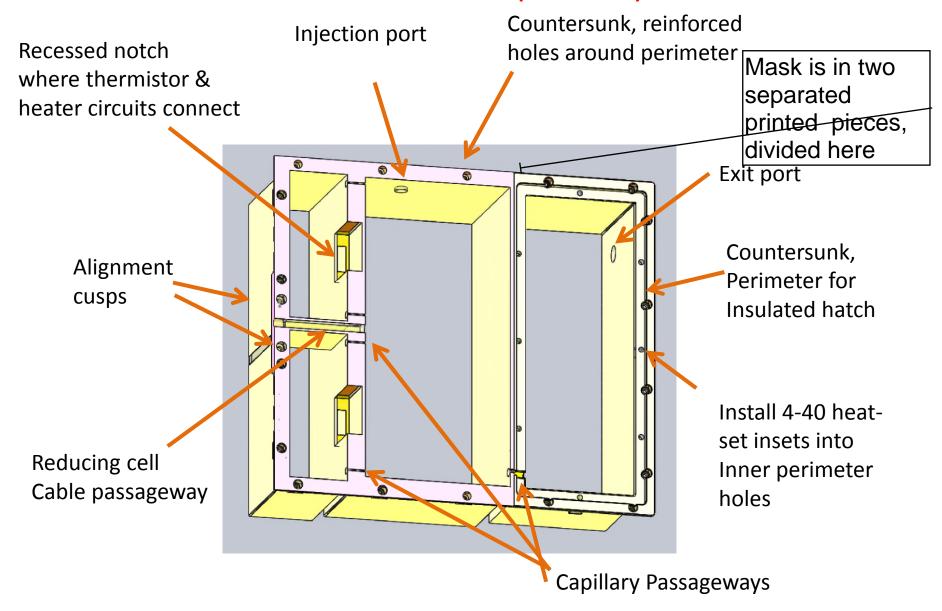
#### View of Shell Assembled with Frame Components



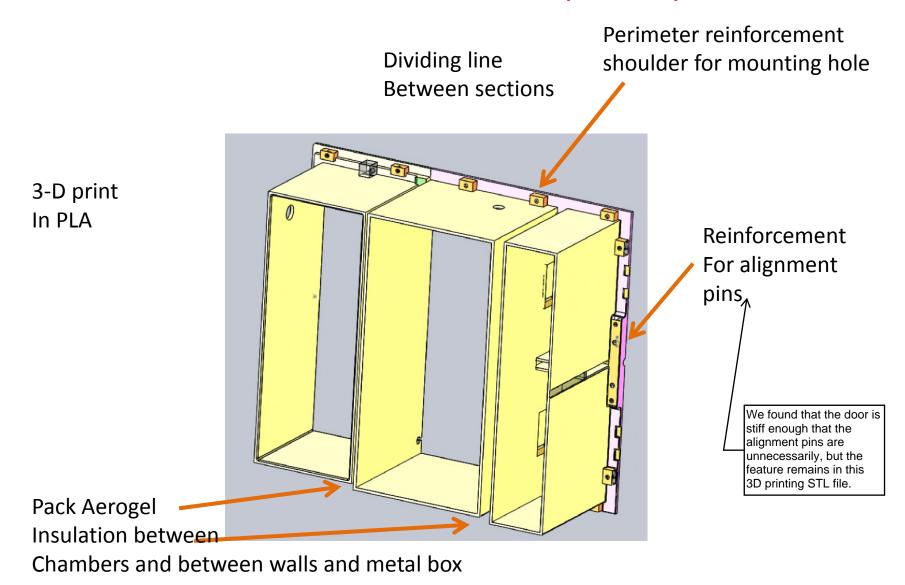
#### HPLC Box with Masks Installed

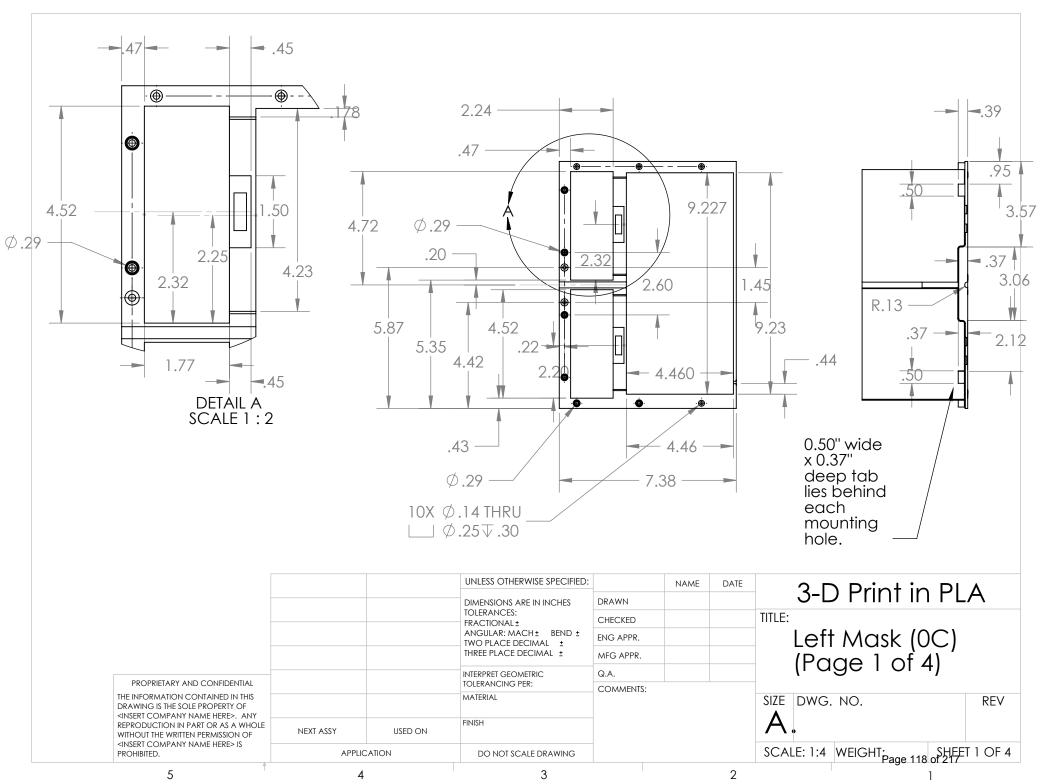


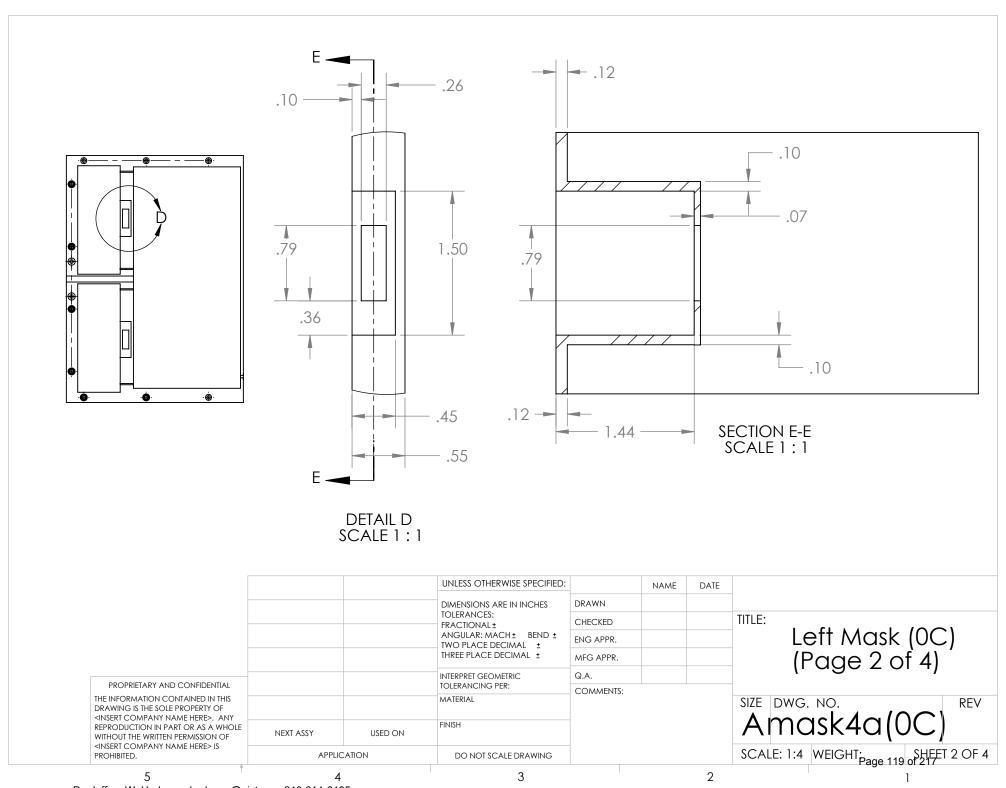
### HPLC Chamber Masks (front)

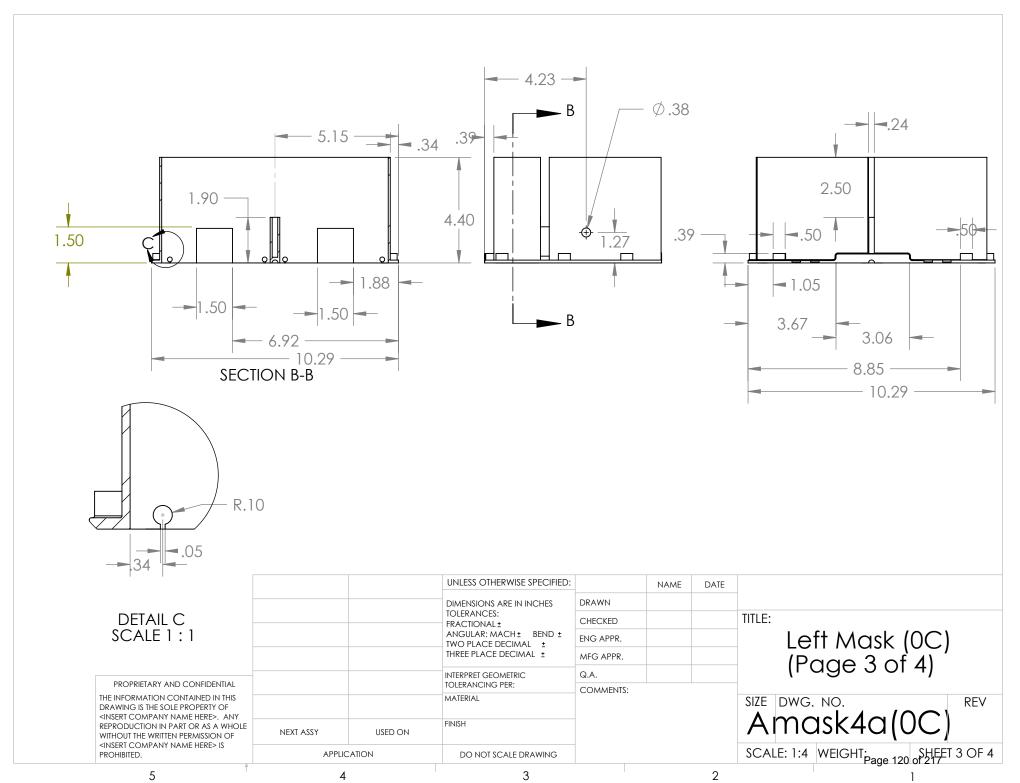


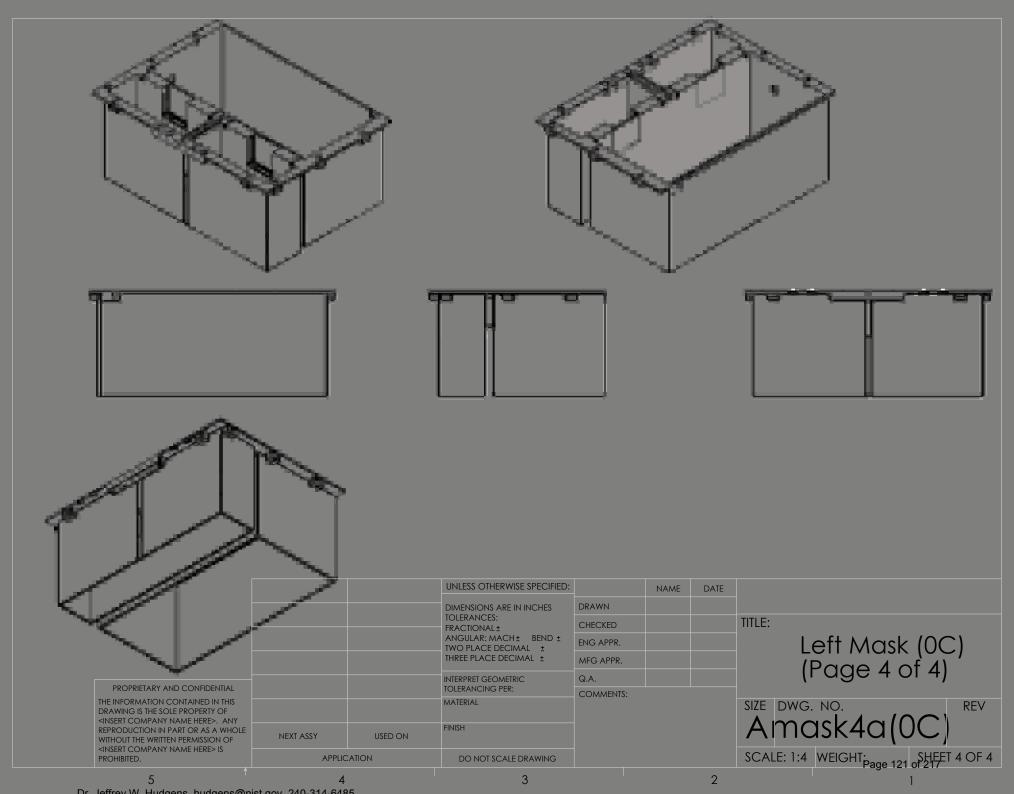
## HPLC Chamber Masks (rear)

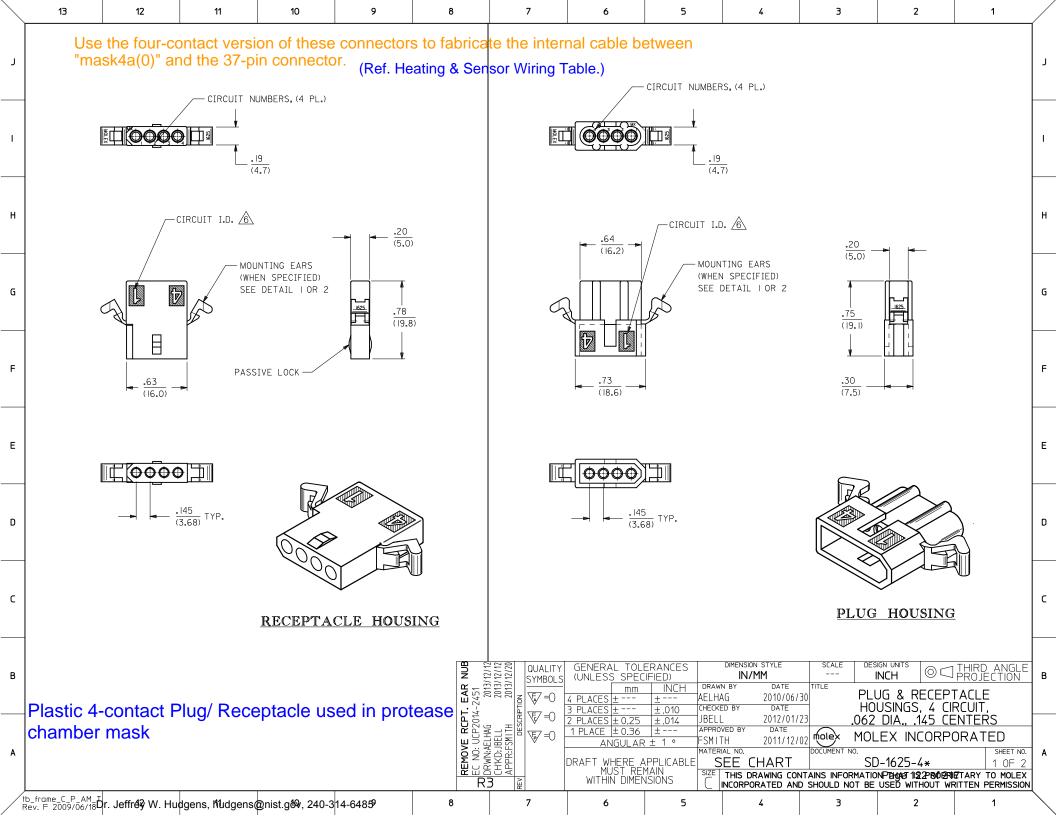


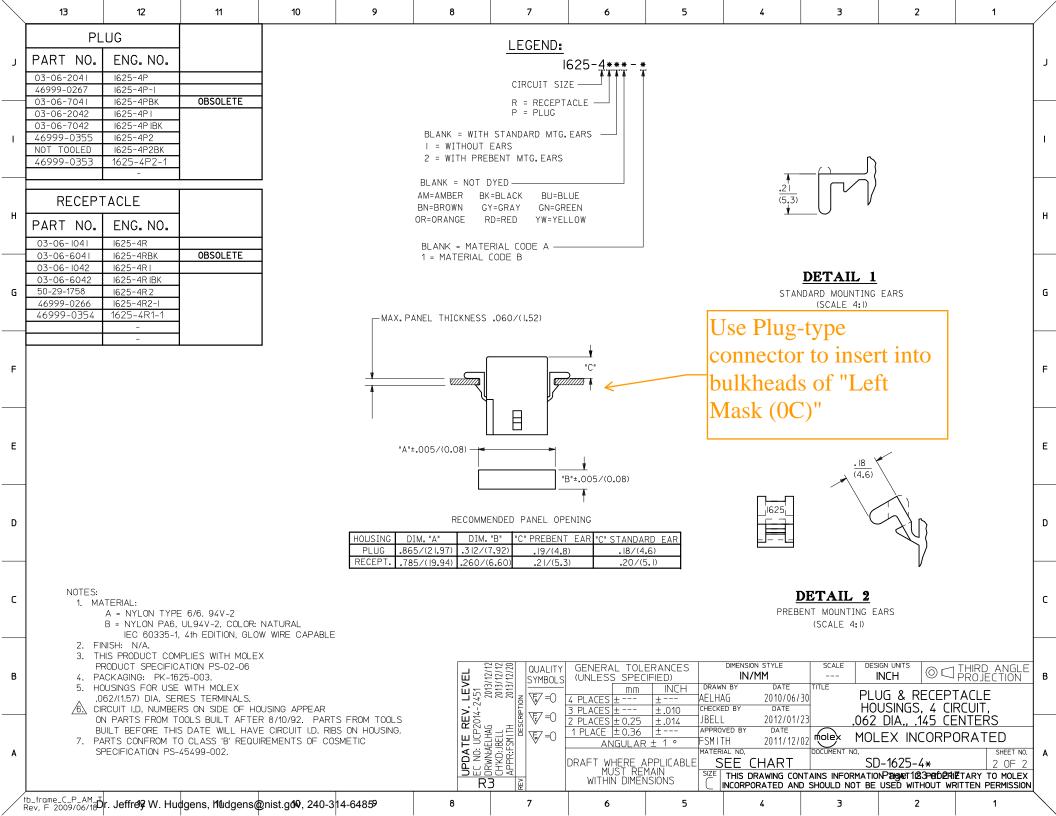


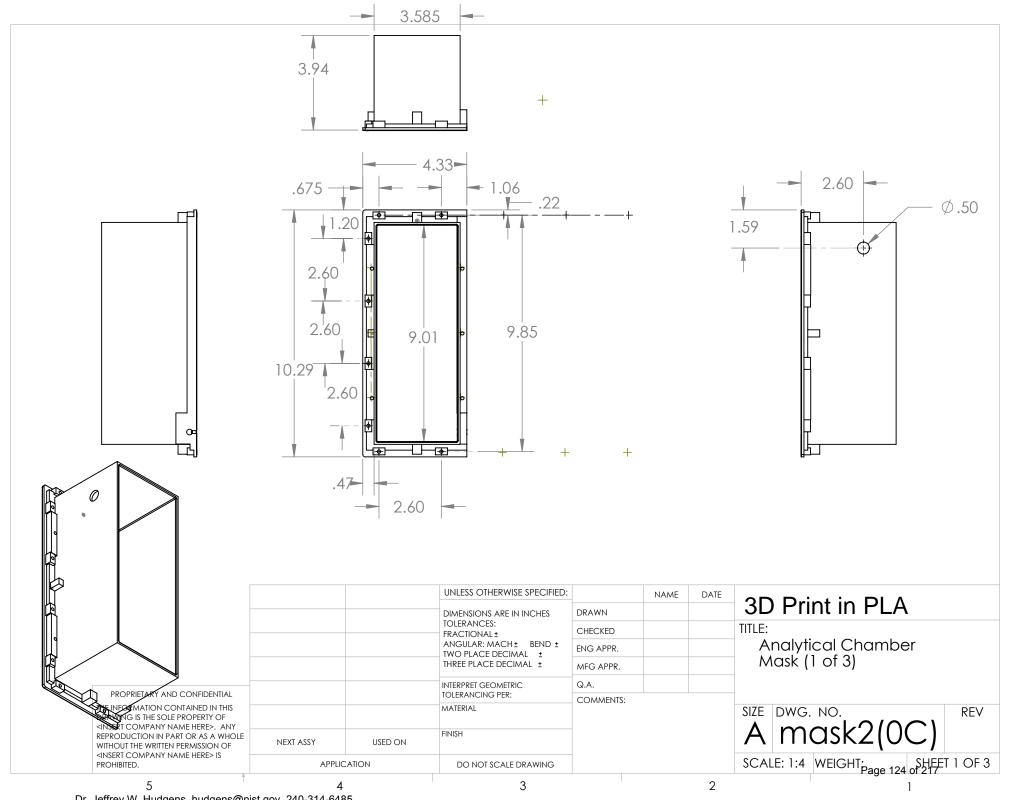




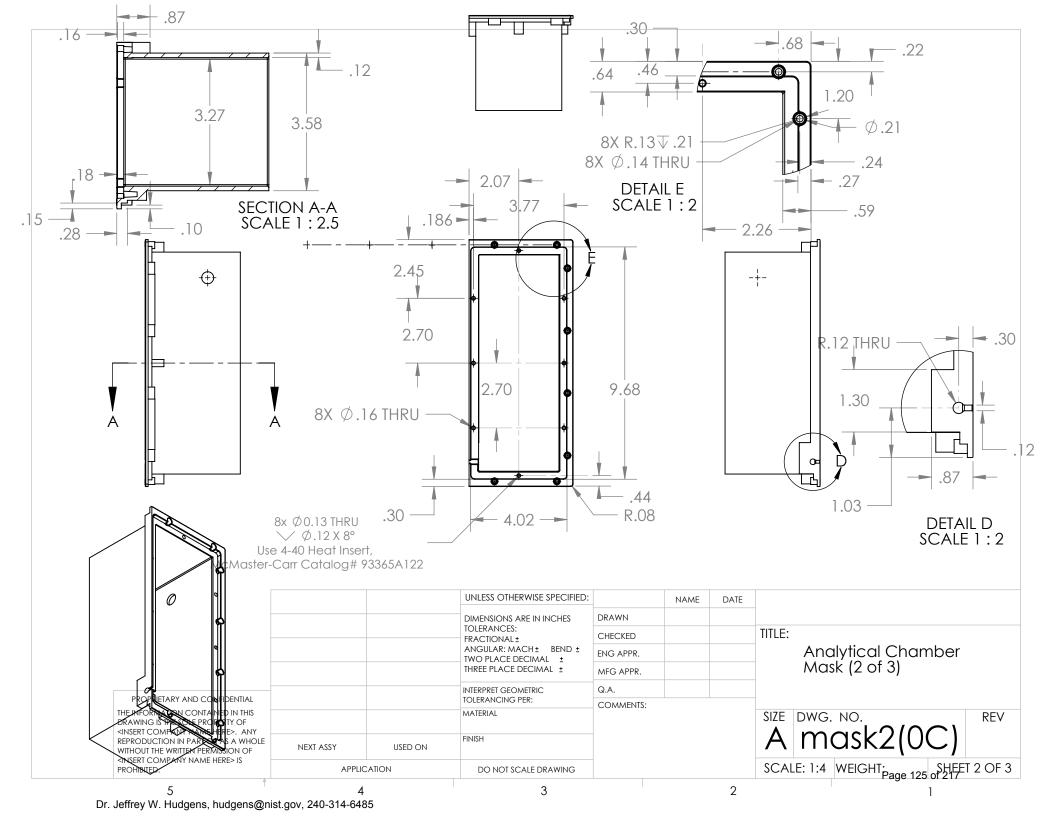


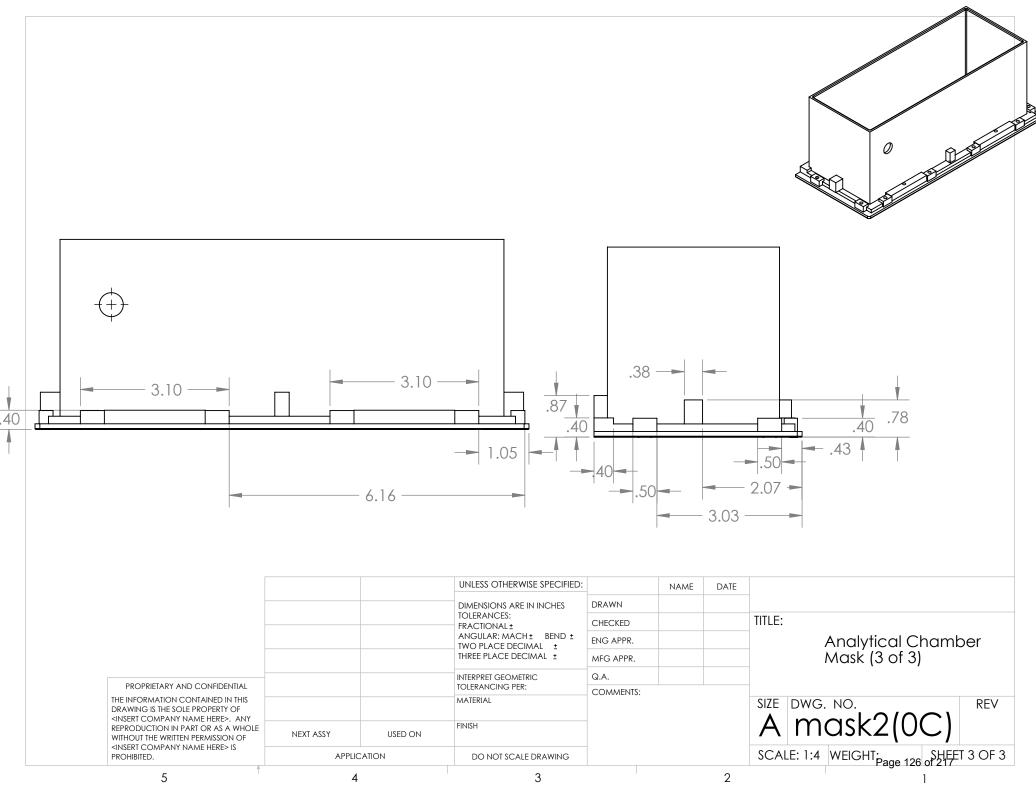




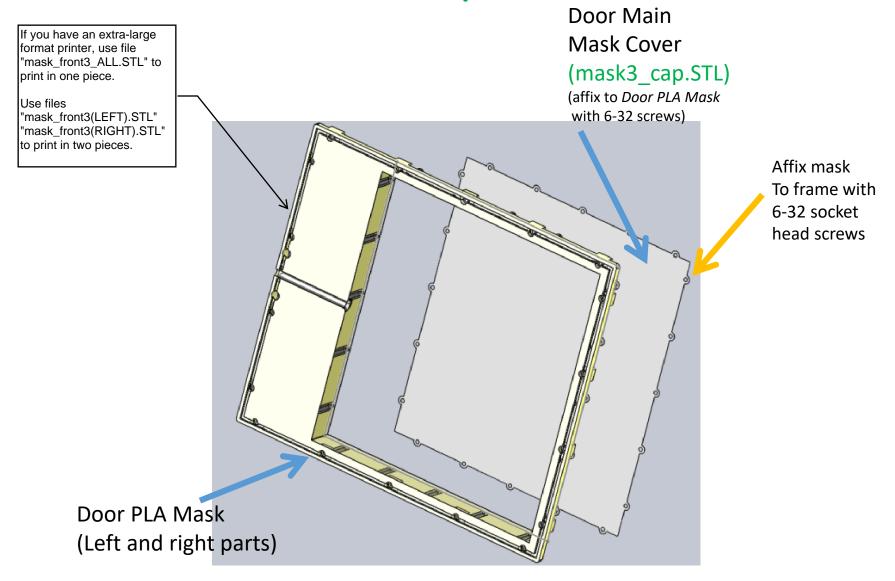


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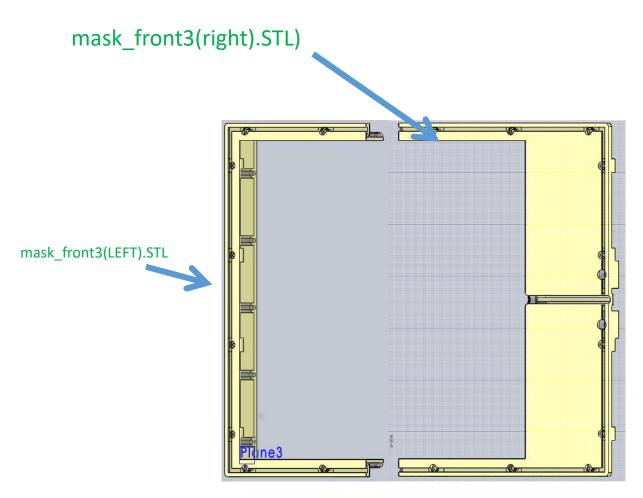




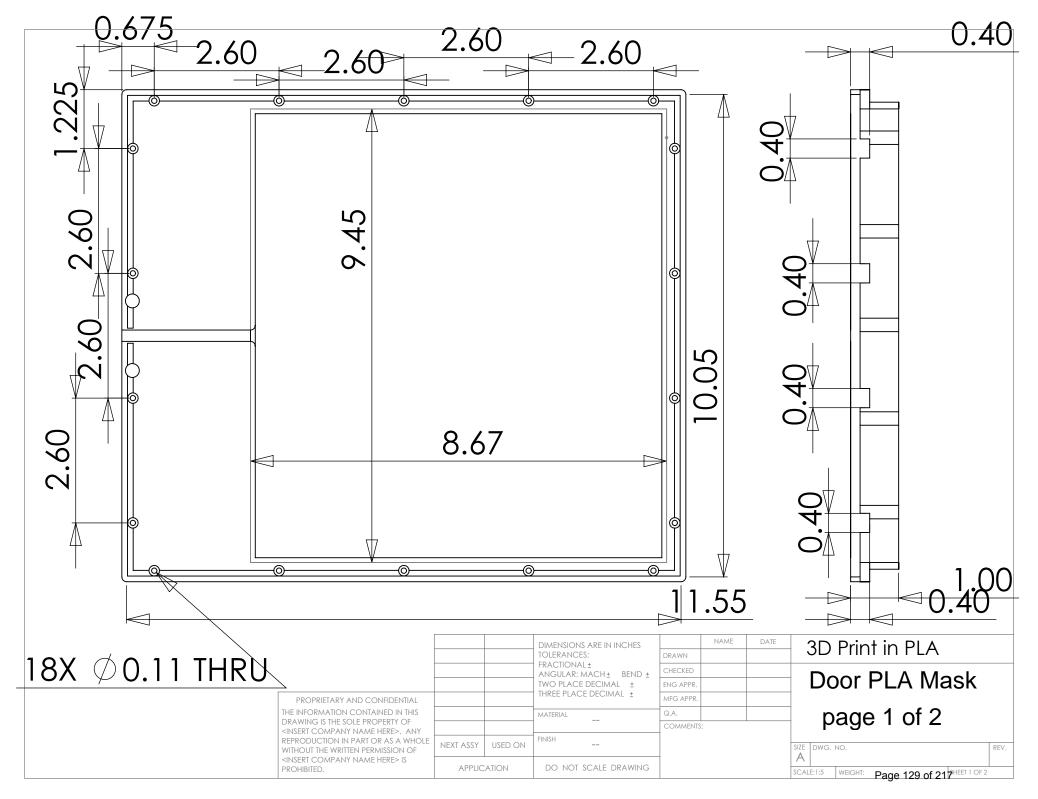
### **Door Mask Assembly**

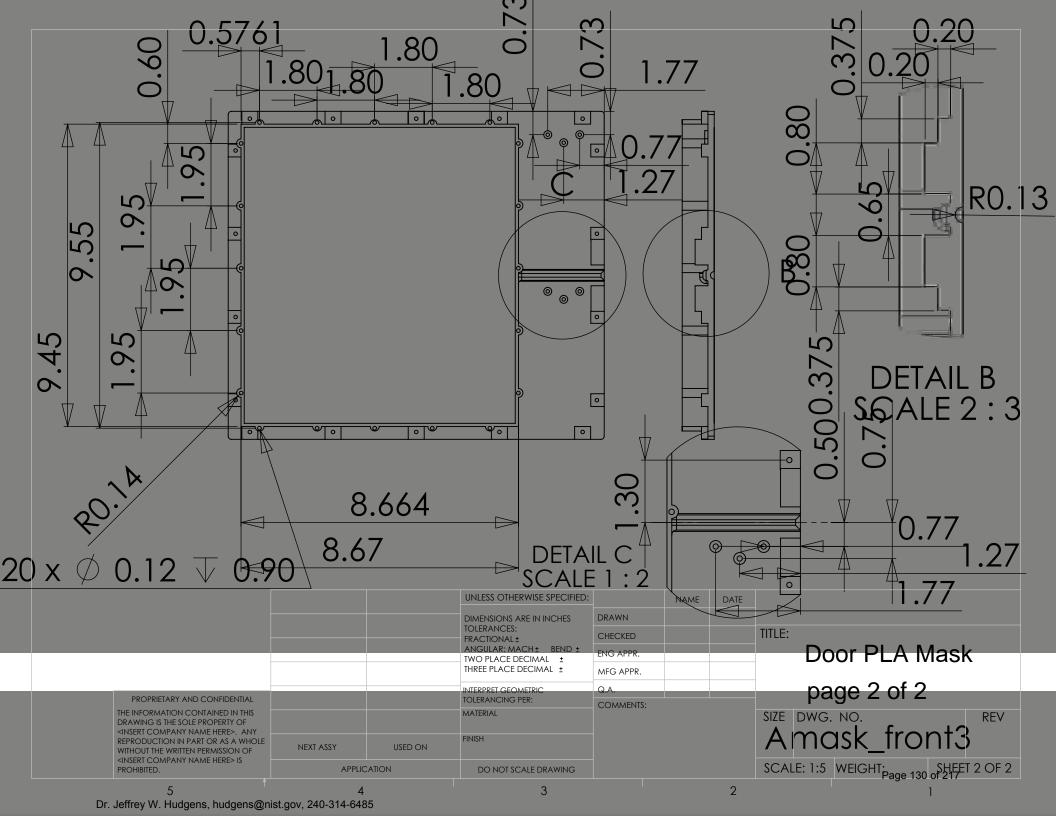


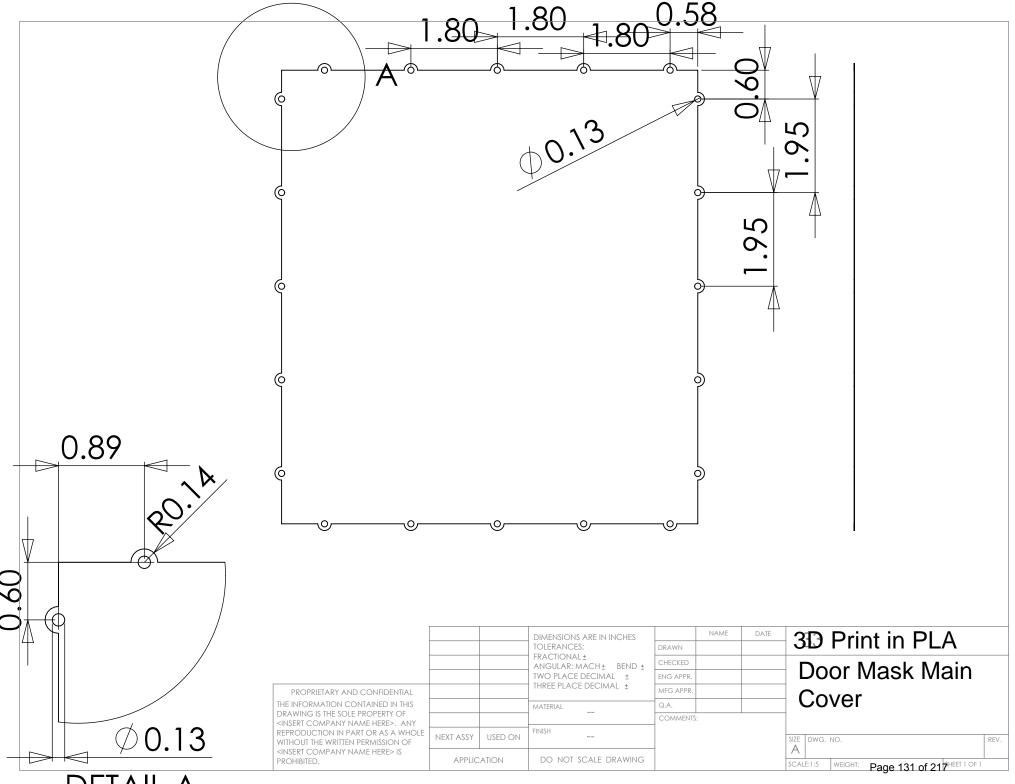
### Door Mask Frame-Mounting Parts



**NOTE:** The above STL files will fit the platens of most medium format (11"x11") 3D printers. A Larger Format Printer may print the file: mask\_front3\_ALL.STL, which contains the left and right parts together.

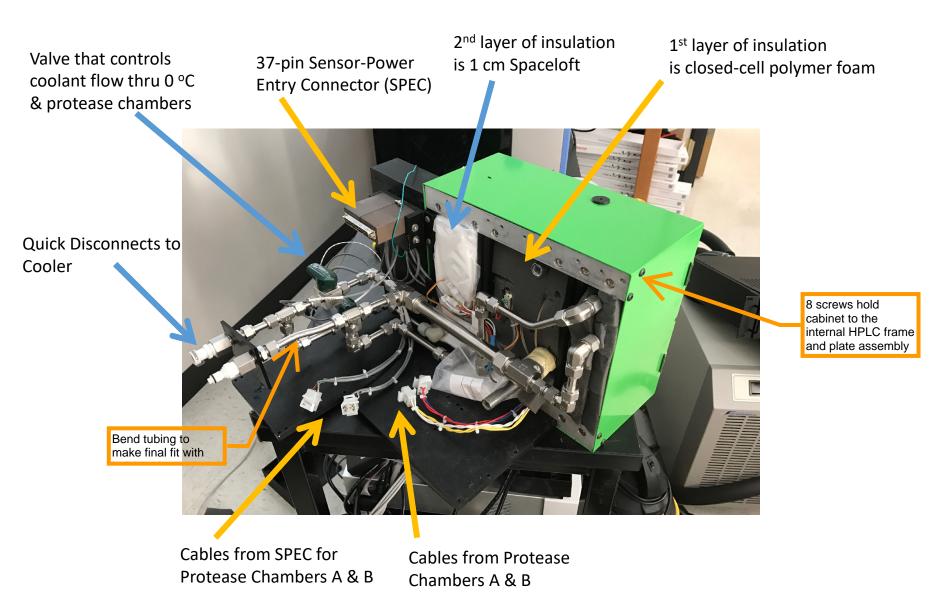


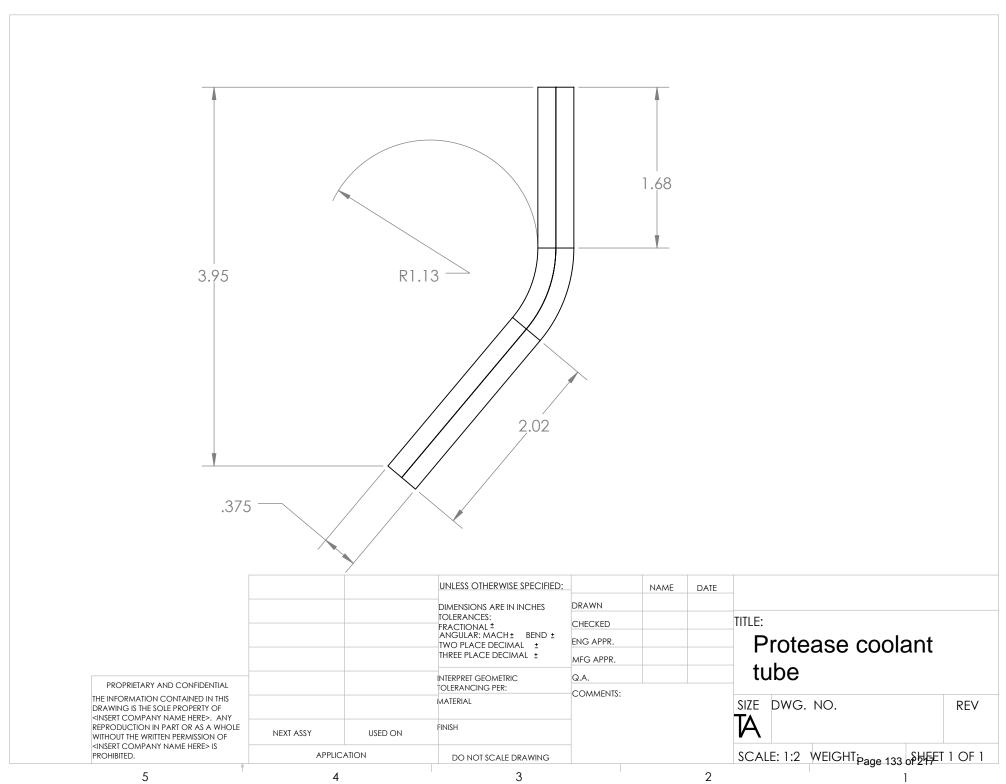




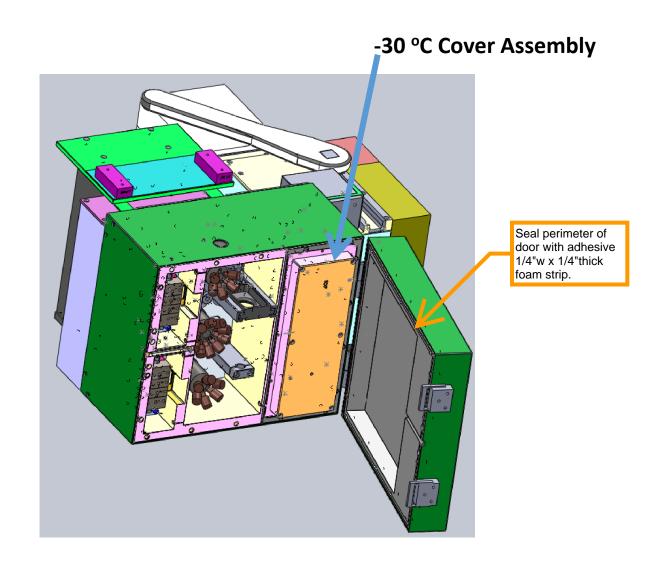
DETAIL Ludgens, hudgens@nist.gov, 240-314-6485

#### View of Cooling and Electrical System



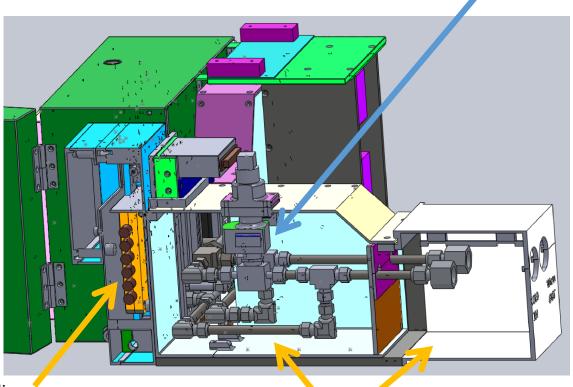


#### HPLC Box with -30 °C Cover



### **Cross-section View Showing Coolant System**

Coupling that actuates throttle valve

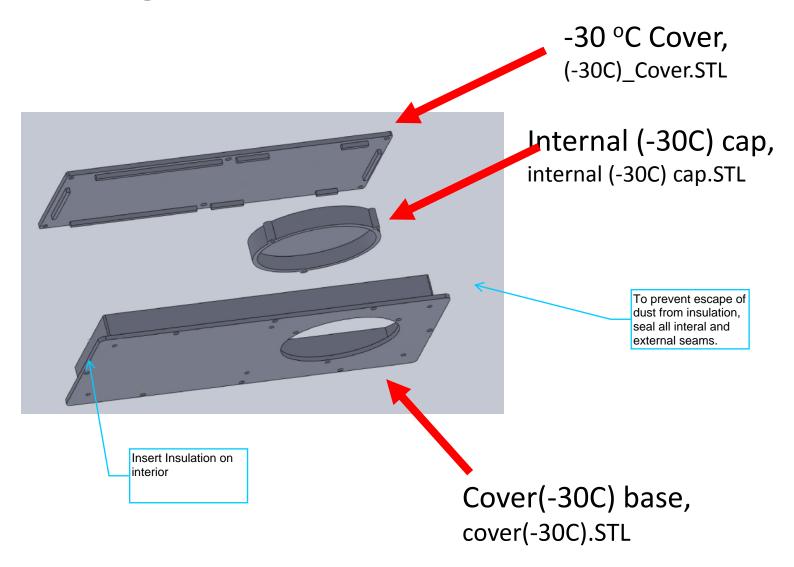


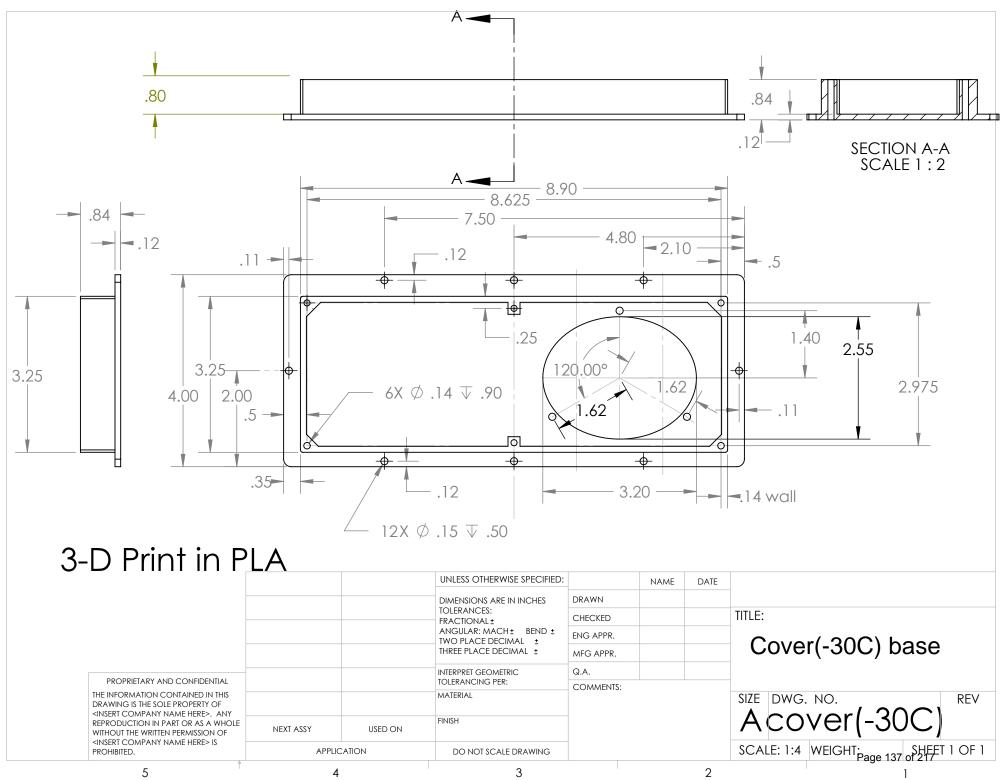
Rack that holds couplings where HPLC fluids enter analytical circuits

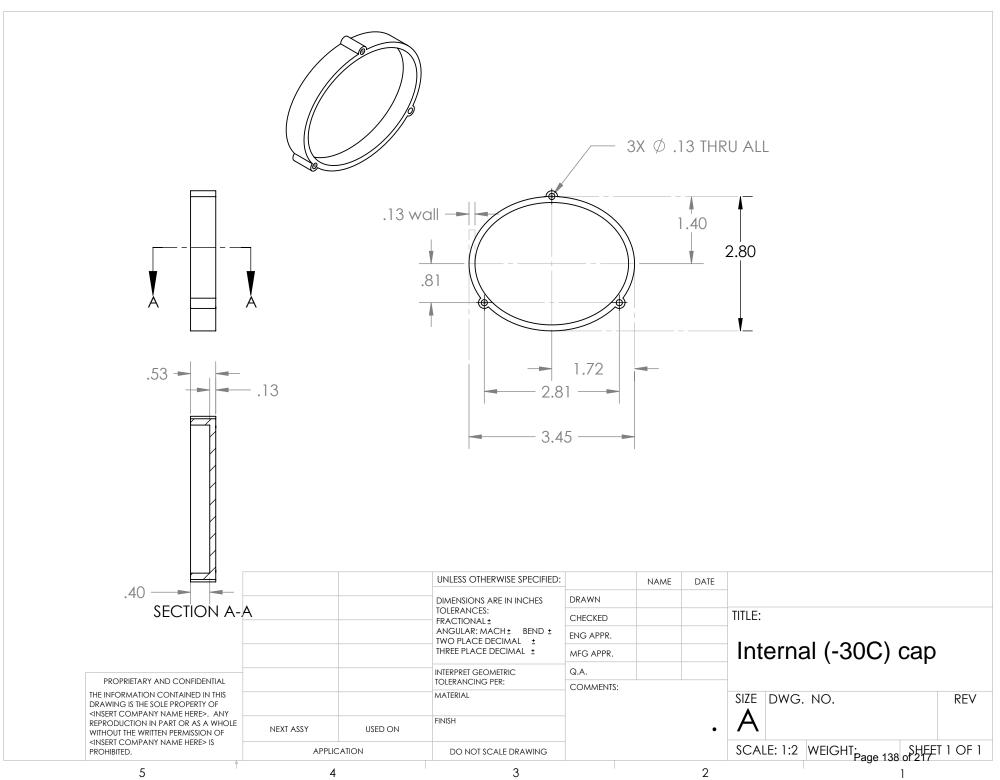
Pack chambers with insulation

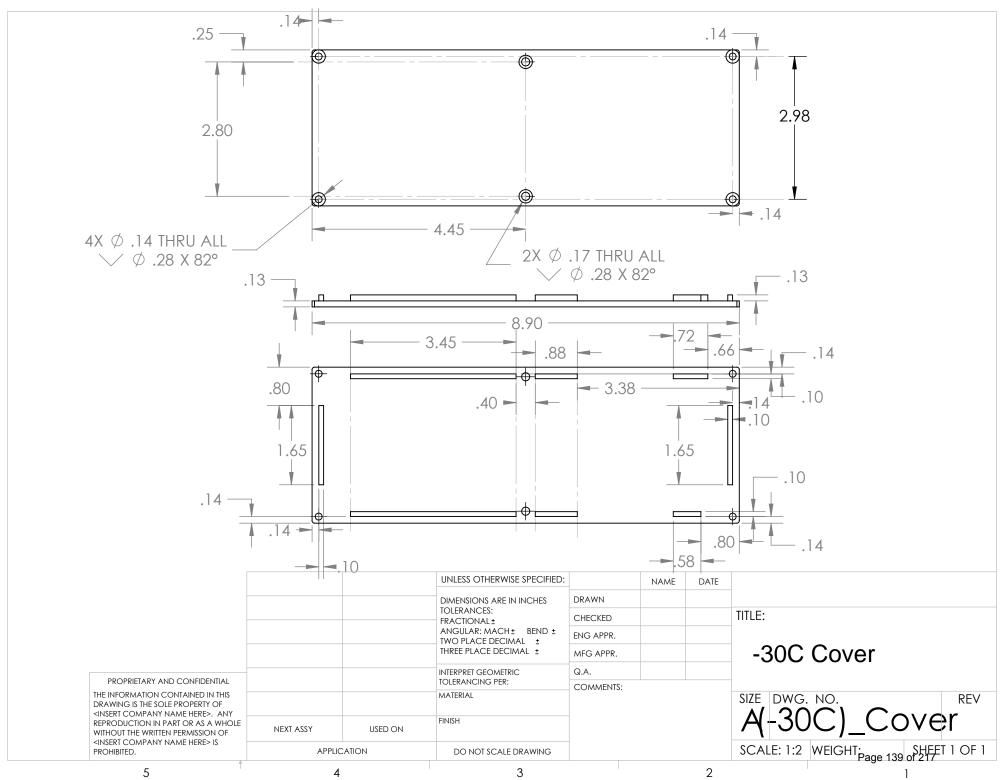
#### Assembly of Internal -30 °C Cover

-- Catalog of STL files

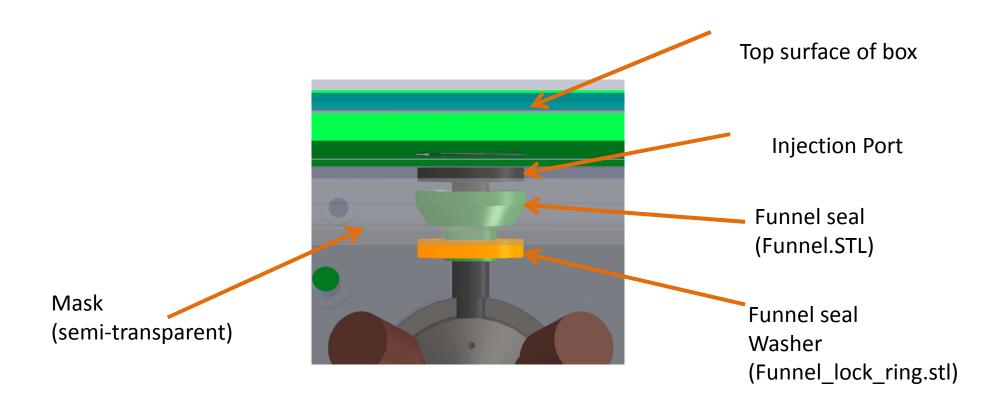


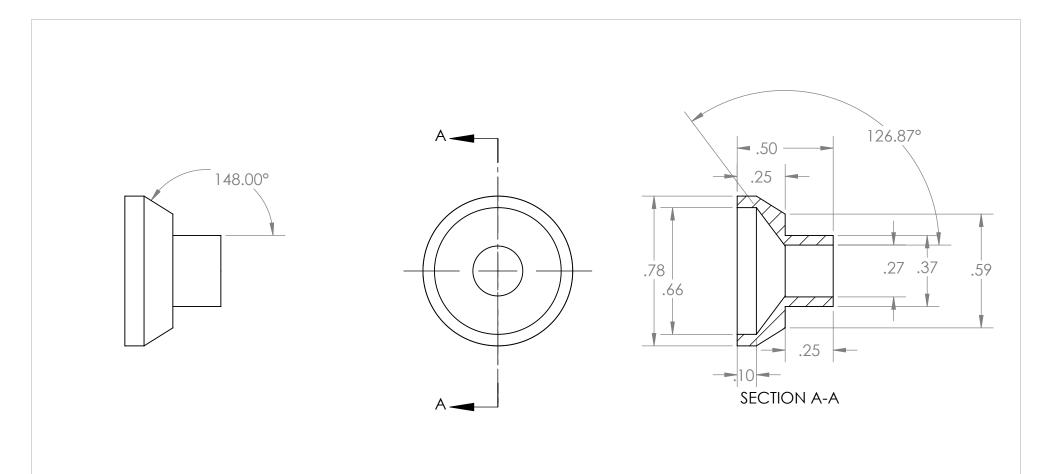






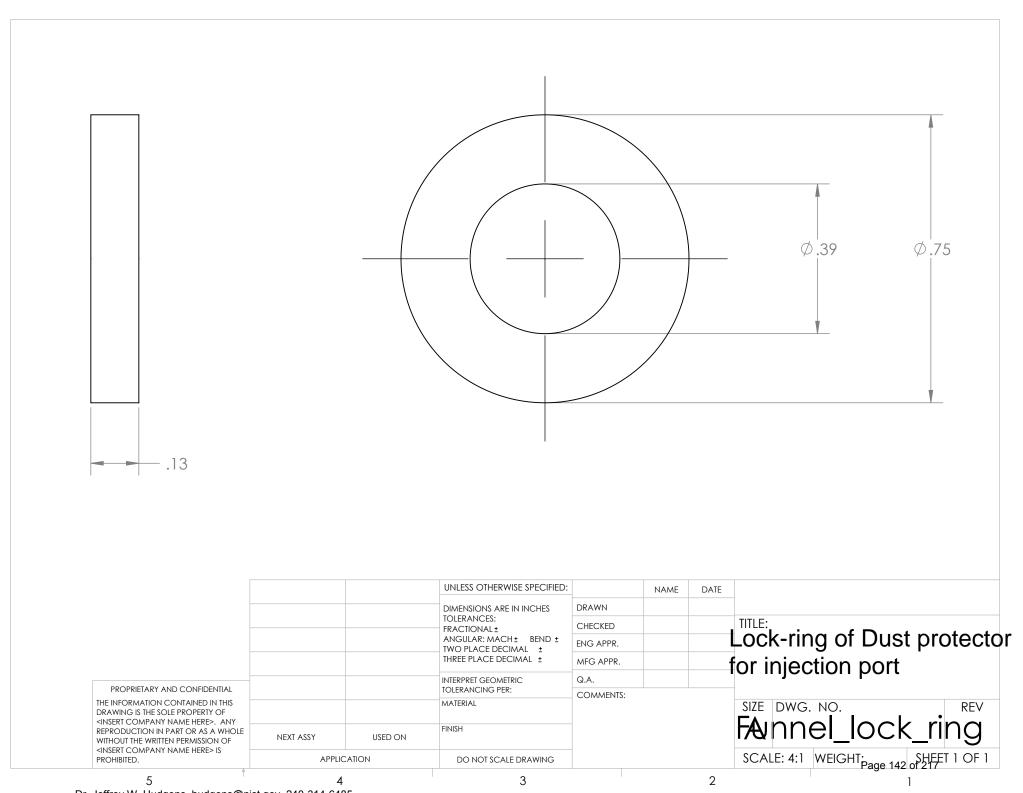
# Sealing Assembly at Injection Port



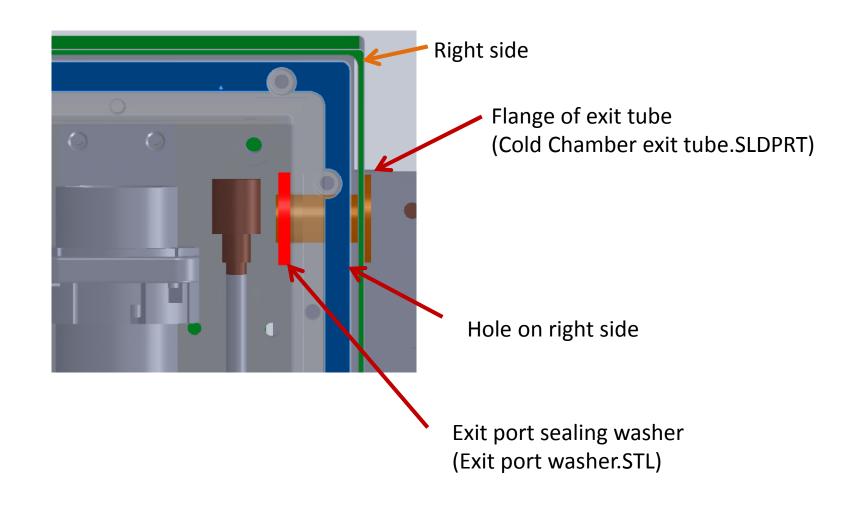


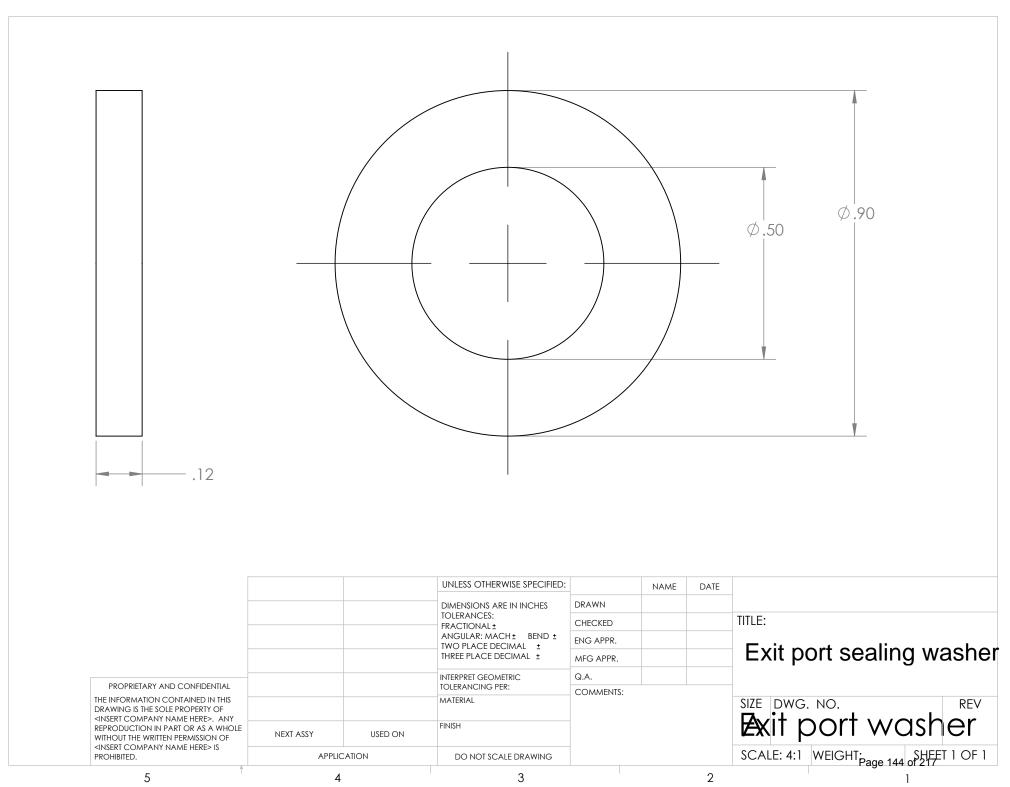
PROPRIETARY AND CONFIDENTIAL THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF <insert company="" here="" name="">. ANY REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF</insert>			UNLESS OTHERWISE SPECIFIED:  DIMENSIONS ARE IN INCHES		NAME	DATE	2D Drint in DLA	
				DRAWN			3D Print in PLA  TITLE:  Dust protector for	
			TOLERANCES: FRACTIONAL ±	CHECKED				
			ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ± INTERPRET GEOMETRIC	ENG APPR.				
				MFG APPR.			•	
				Q.A.			injection port	
			TOLERANCING PER:  MATERIAL	COMMENTS:			SIZE DWG. NO. REV	
	NEXT ASSY	USED ON	FINISH	-			A Funnel	
<insert company="" here="" name=""> IS PROHIBITED.</insert>	APPLICATION		DO NOT SCALE DRAWING				SCALE: 2:1 WEIGHT: SHEET 1 OF 1	
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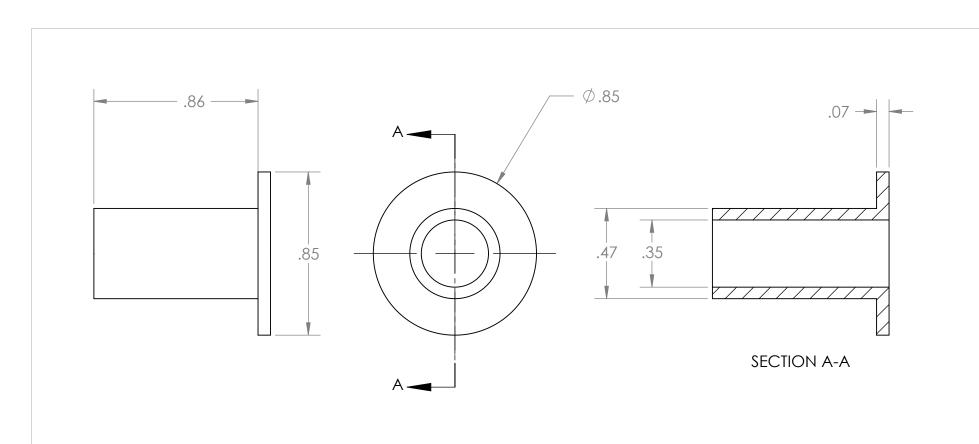
5 4
Dr. Jeffrey W. Hudgens, hudgens@nist.gov, 240-314-6485



## Sealing Assembly at -30 °C Exit Port



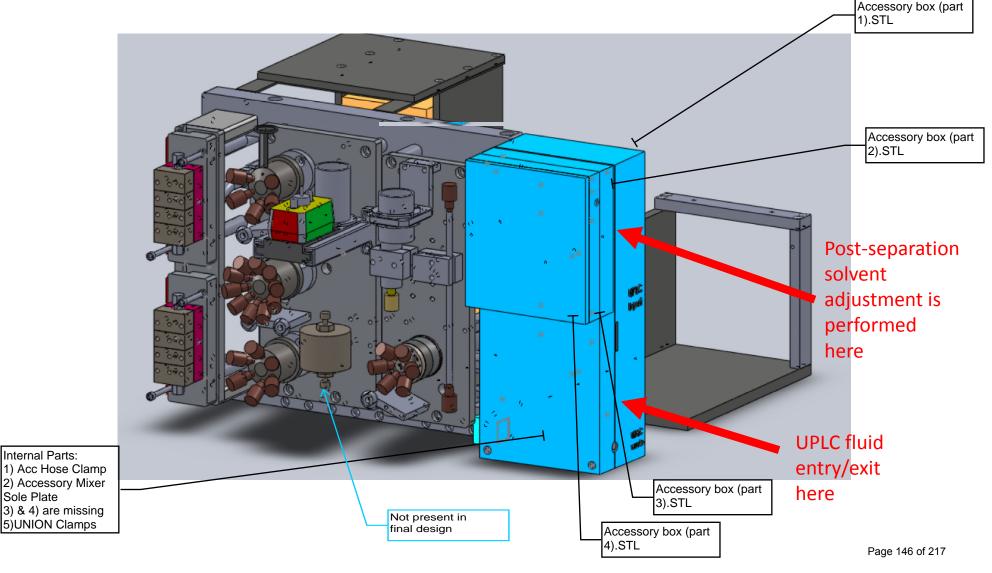




			UNLESS OTHERWISE SPECIFIED:		NAME	DATE				
PROPRIETARY AND CONFIDENTIAL THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF <insert <insert="" a="" any="" as="" company="" heres="" heres.="" in="" is<="" name="" of="" or="" part="" permission="" reproduction="" td="" the="" whole="" without="" written=""><td></td><td></td><td>DIMENSIONS ARE IN INCHES</td><td>DRAWN</td><td></td><td></td><td></td><td></td><td></td><td></td></insert>			DIMENSIONS ARE IN INCHES	DRAWN						
			TOLERANCES: FRACTIONAL ±	CHECKED			Cold Chamber exit tu			
			ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±  INTERPRET GEOMETRIC	ENG APPR.						hu ib o
				MFG APPR.						lube
				Q.A.						
			TOLERANCING PER:  MATERIAL	COMMENTS:			SIZE DWG. NO. REV			REV
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PROHIBITED.	APPLICATION		DO NOT SCALE DRAWING				SCALE: 2:1	WEIGHT: Page 145	SHEET OF 217	I OF 1
5	4		3			2			1	

# Populated Internal Frame (STL File Directory)

(Entire assembly slides into box as one piece)



## STL Catalog Housing for NIST HPLC, part 1

Backside housing top metal (left--back).SLDPRT

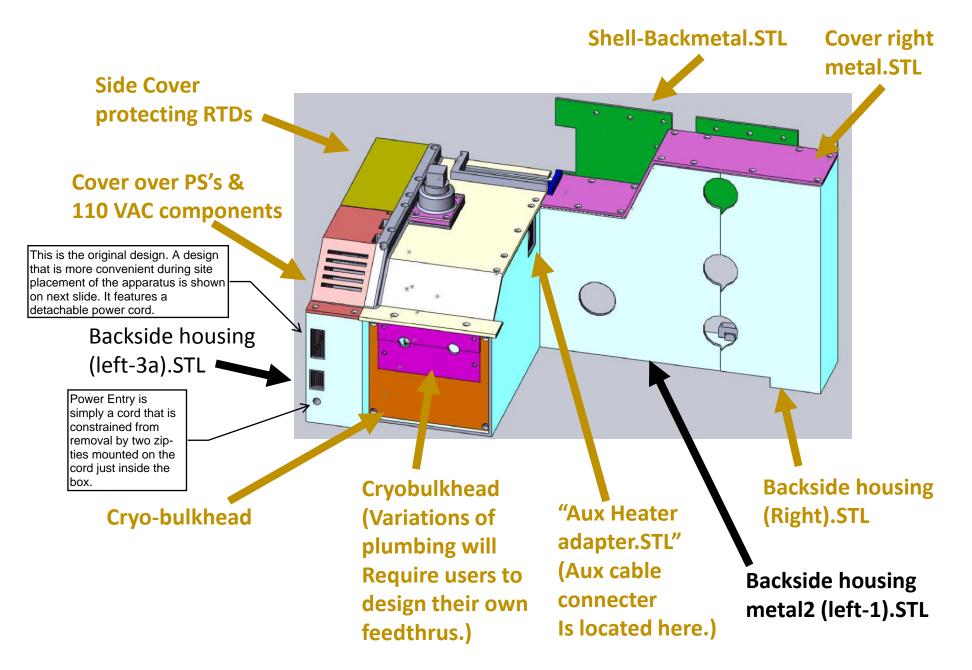
#### **Housing Installation:**

- Start with no components on "motor rail base"
- Install "Shell-back metal.STL" (Screw holes are also provide to flatten panel against aluminum extrusions.)
- Install "Backside housing (left-3a).STL" and "Backside housing metal (left-2).STL"
- 4) Push "Backside housing metal2 (left-1).SLDPRT" along the assembly in #3 to directly engage the clamp. The assembly may be further stabilized with a 6-32 cap-head screw.
- 5) Push "Backside housing (right).SLDPRT" in high to allow clamps to engage with "Backside housing metal2 (left-1).SLDPRT". (You may want to rotate this into place.) Affix to Al frame using a single 8-32 screw.

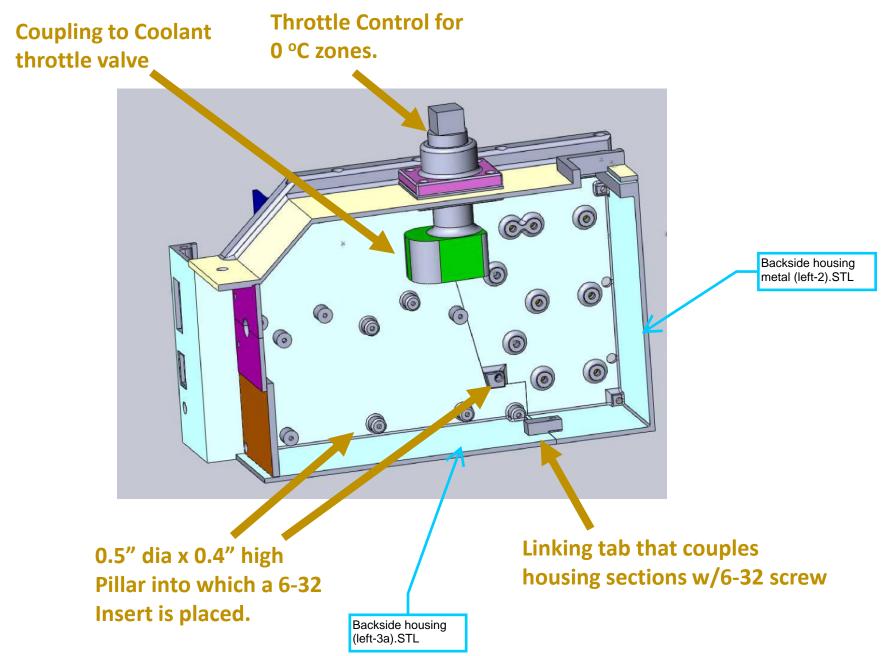
Valve adjust thotler valve driver.SLDPRT Backside sleeve.STL (Adjust with Wrench.STL) housing top metal (left--back).STL Side Cover.SLDPRT **Backside housing** (left-4).STL **Backside housing Backside housing metal** metal2 (left-1).STL (left-2).SLDPRT

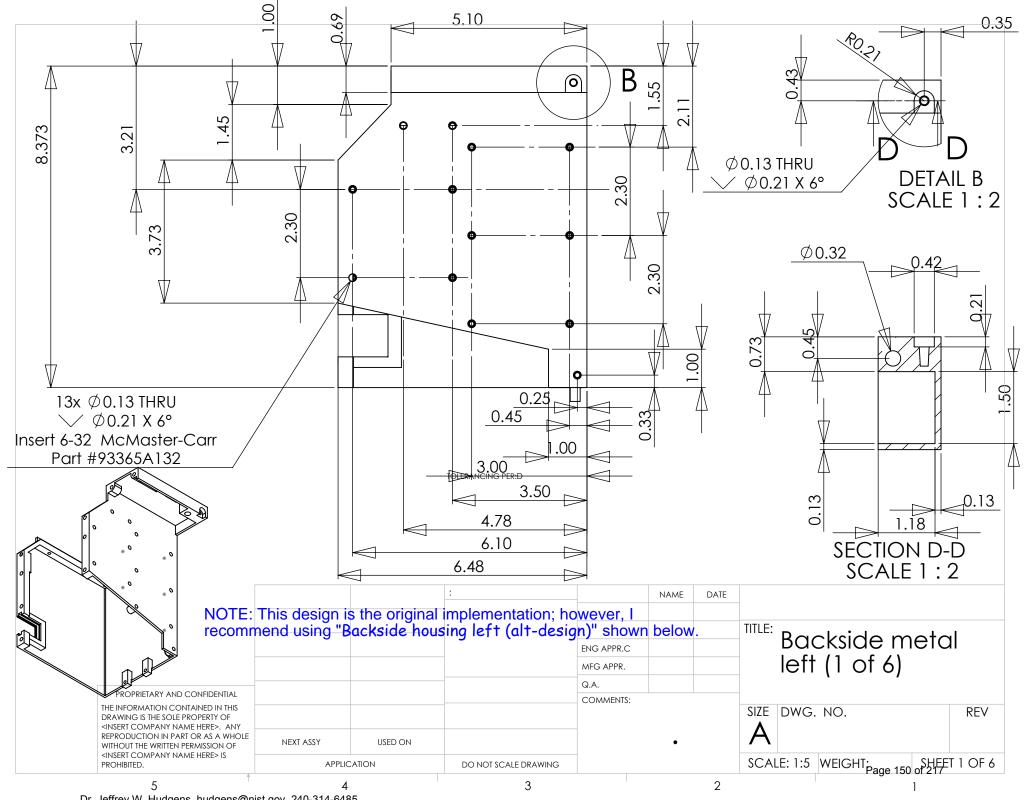
Hooks are engaged by rotating "Backside housing (Right).STL" into position.

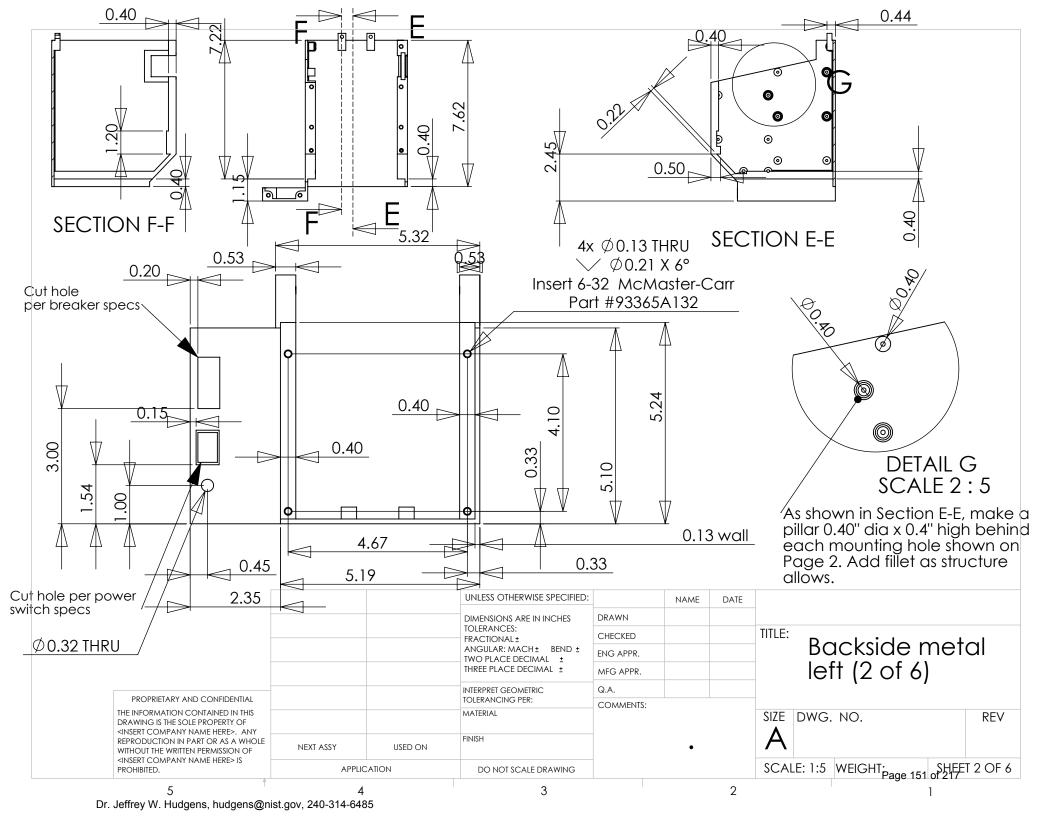
#### STL Catalog Housing for NIST HPLC, part 2

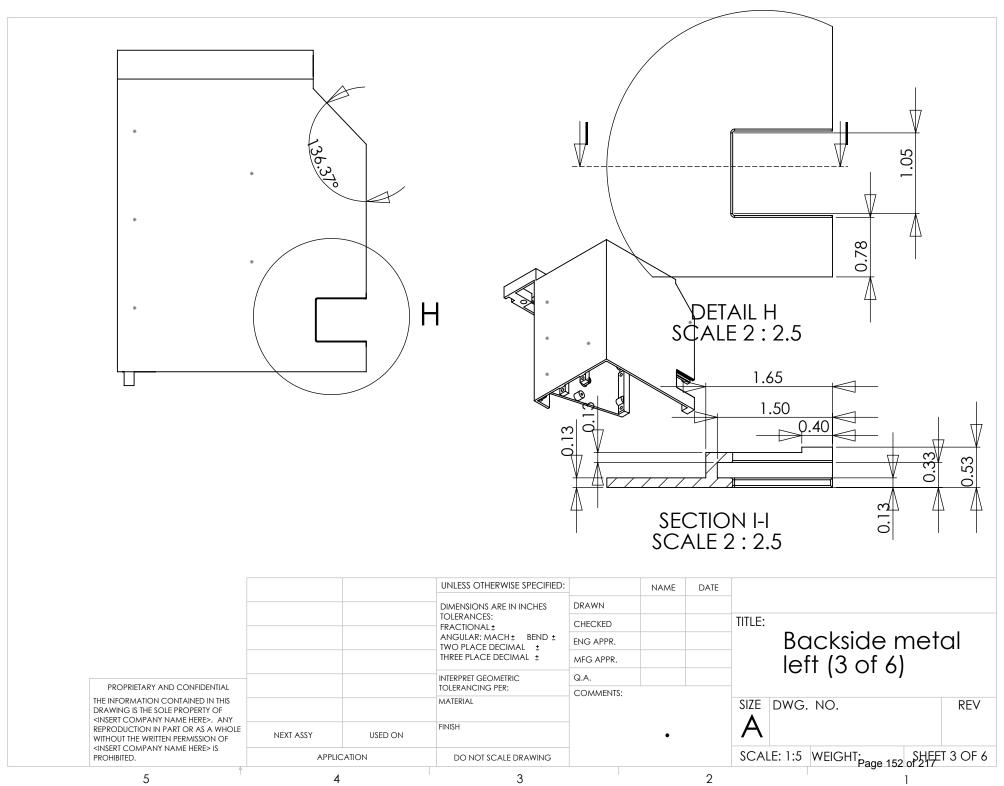


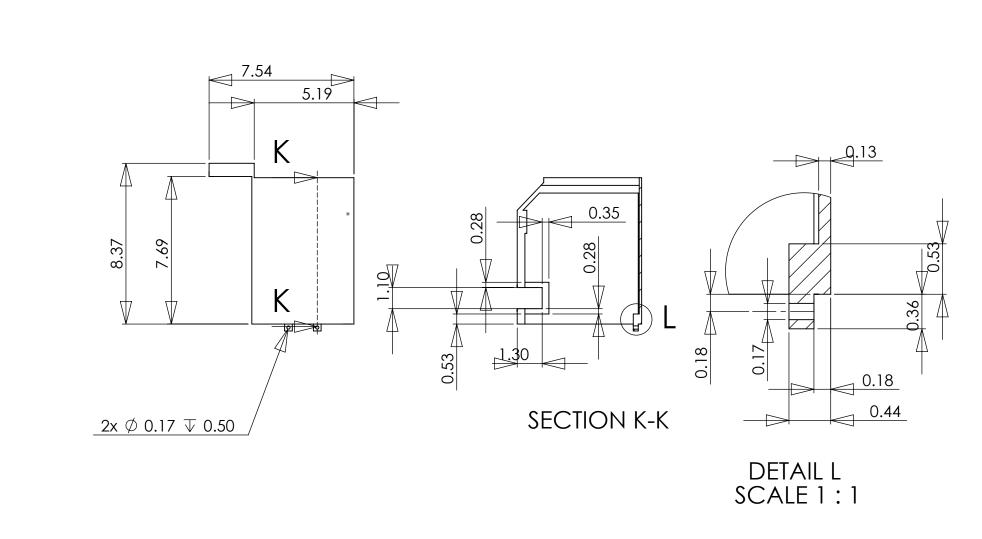
### Cut-away of Housing Near Coolant Mixing Zone



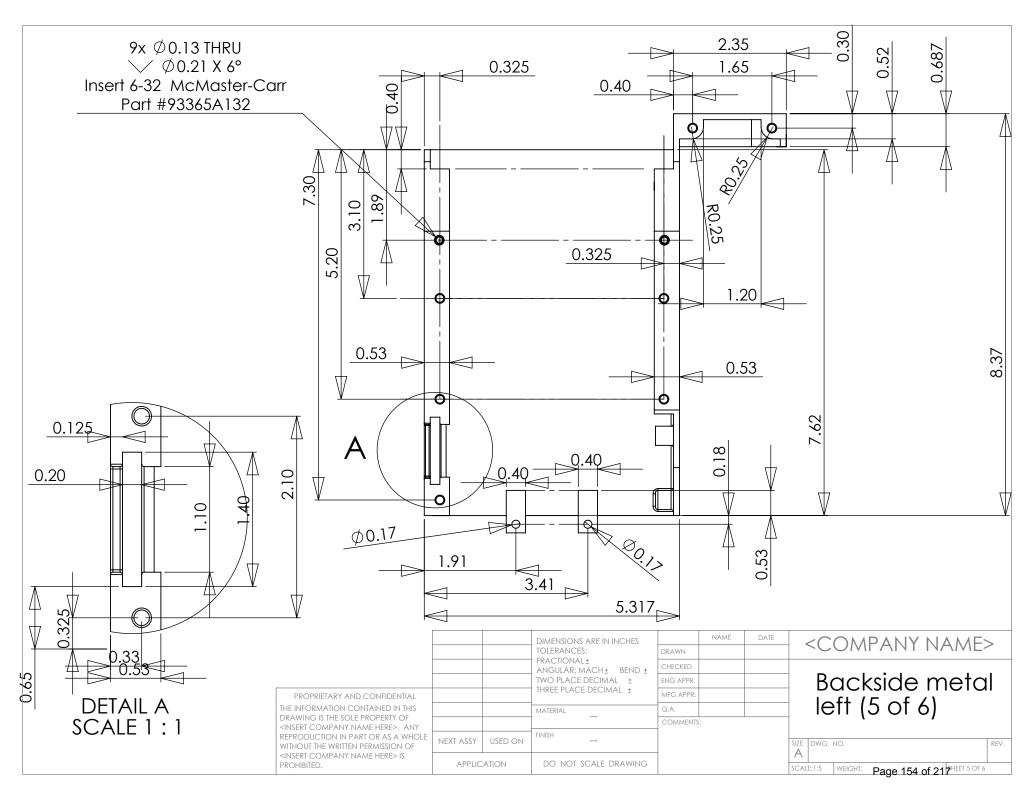


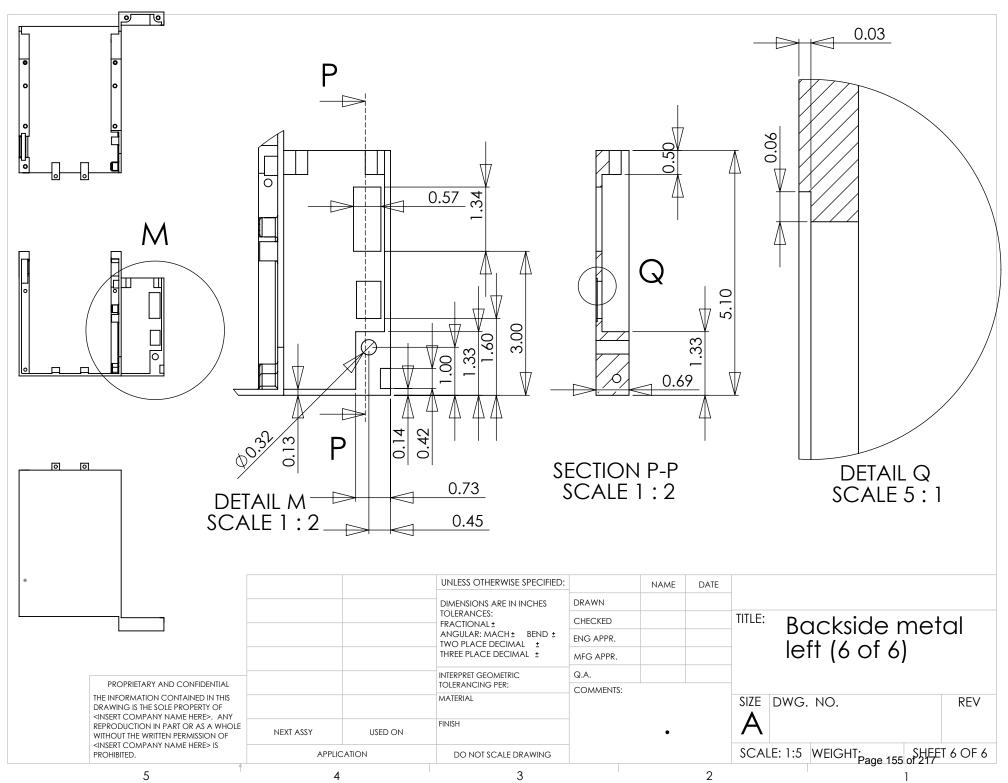




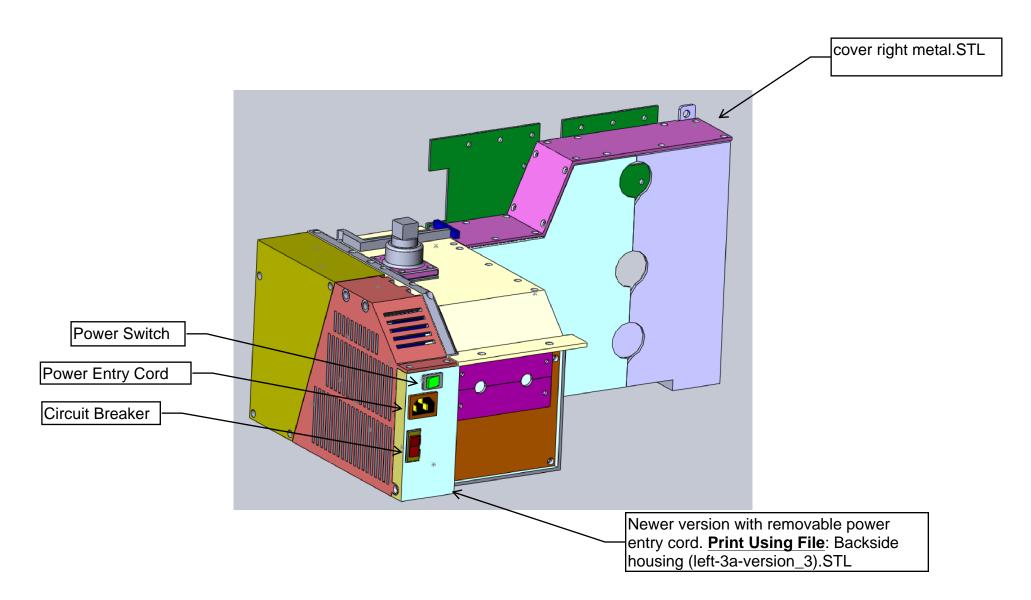


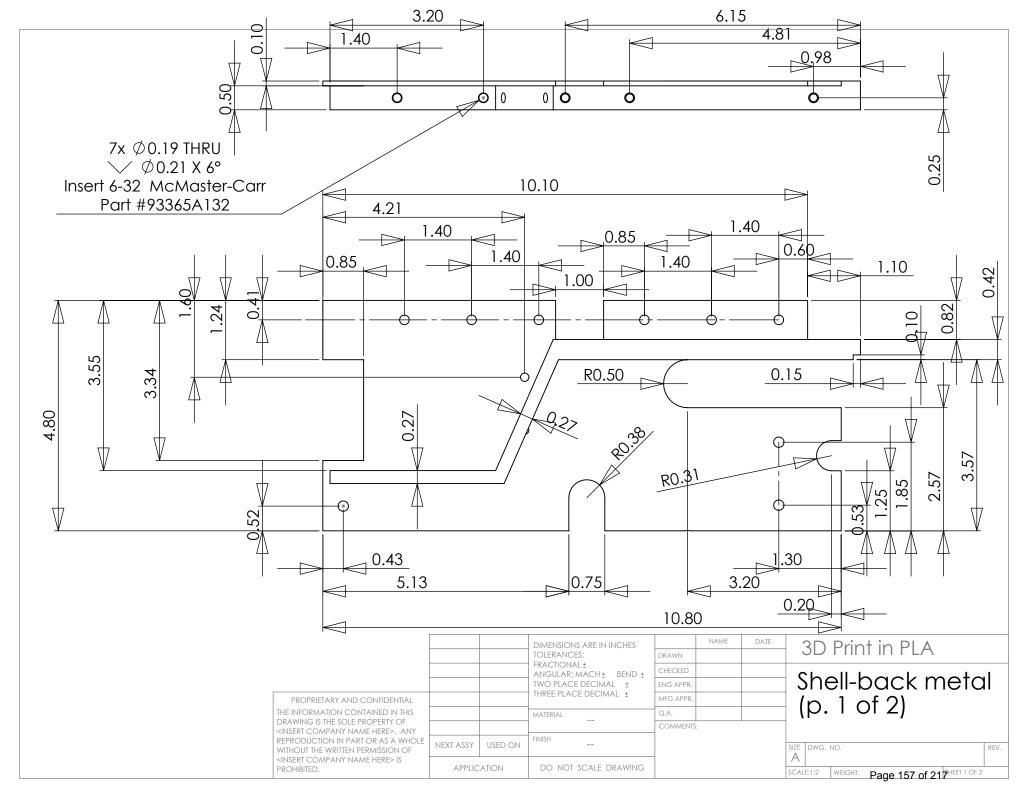
UNLESS OTHERWISE SPECIFIED: NAME DATE DRAWN DIMENSIONS ARE IN INCHES **TOLERANCES:** TITLE: CHECKED FRACTIONAL ± Backside metal ANGULAR: MACH ± BEND ± ENG APPR. TWO PLACE DECIMAL ± left (4 of 6) THREE PLACE DECIMAL ± MFG APPR. Q.A. INTERPRET GEOMETRIC PROPRIETARY AND CONFIDENTIAL TOLERANCING PER: COMMENTS: THE INFORMATION CONTAINED IN THIS MATERIAL SIZE DWG. NO. **REV** DRAWING IS THE SOLE PROPERTY OF <INSERT COMPANY NAME HERE>. ANY FINISH REPRODUCTION IN PART OR AS A WHOLE NEXT ASSY USED ON WITHOUT THE WRITTEN PERMISSION OF <INSERT COMPANY NAME HERE> IS SCALE: 1:5 WEIGHT: SHEET 4 OF 6 APPLICATION PROHIBITED. DO NOT SCALE DRAWING 3 2

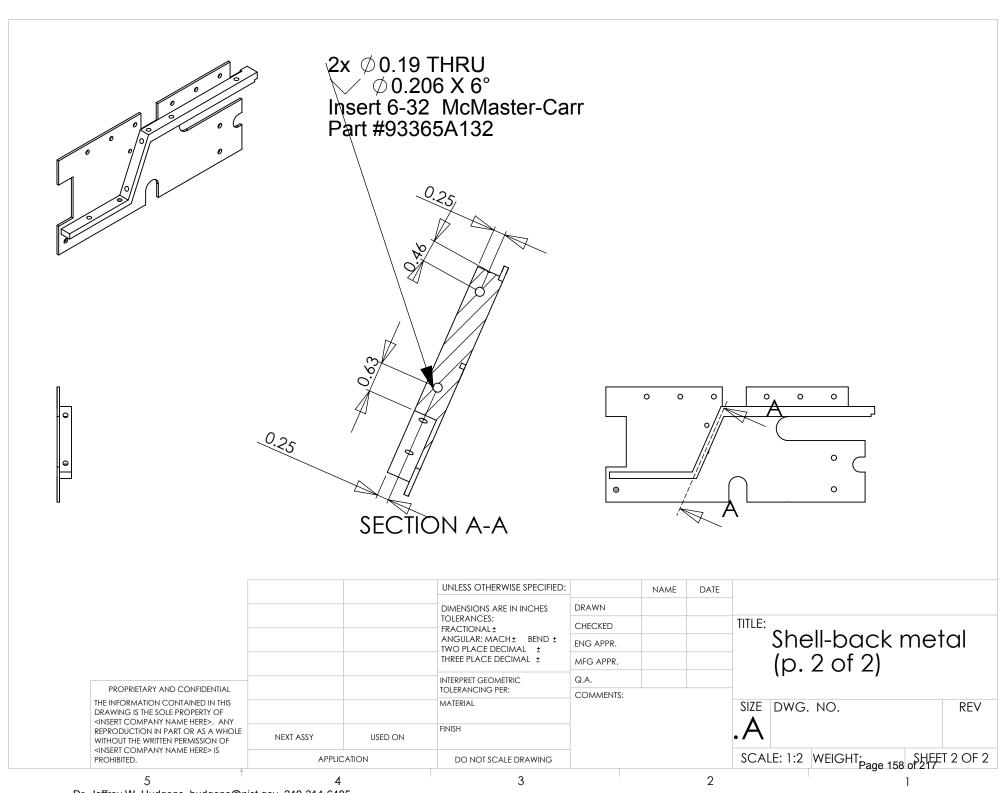




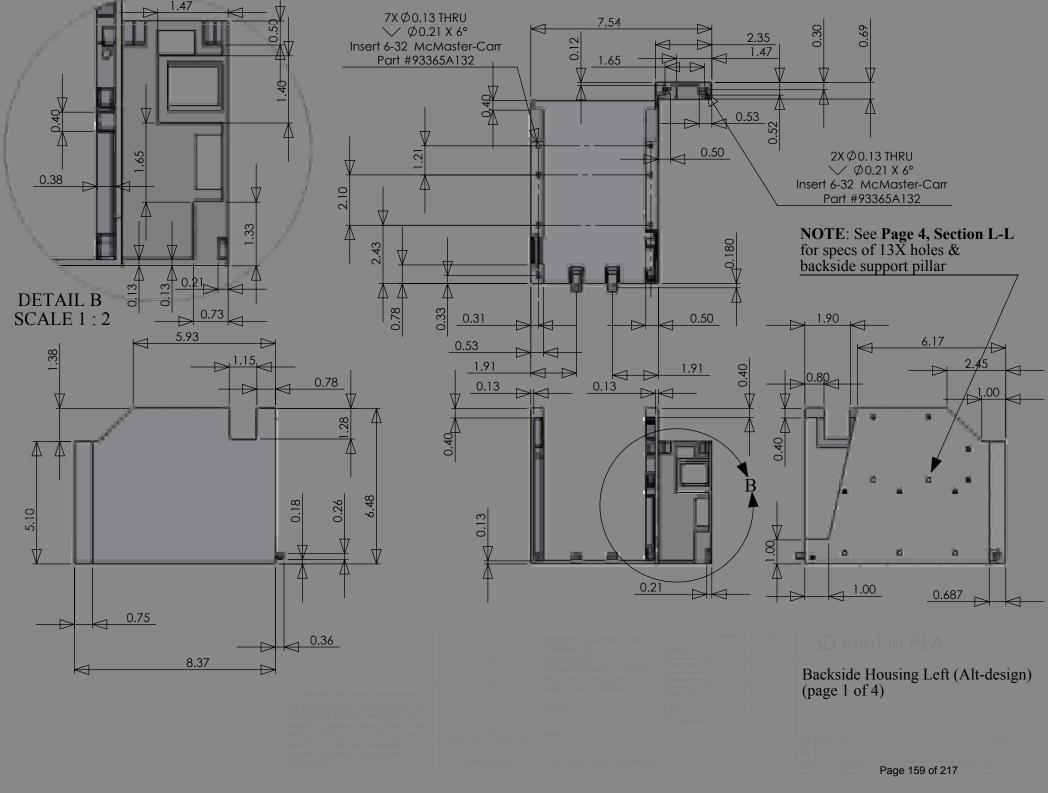
# **Backside Housing Showing Newer Power Entry**

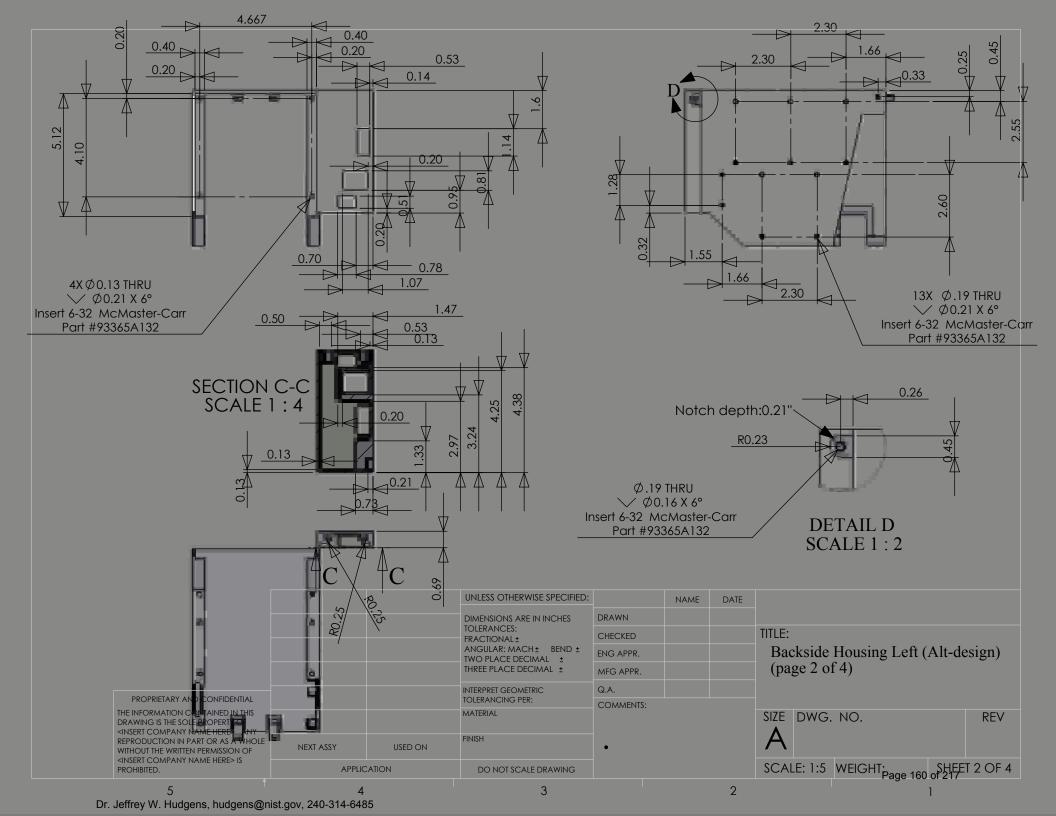


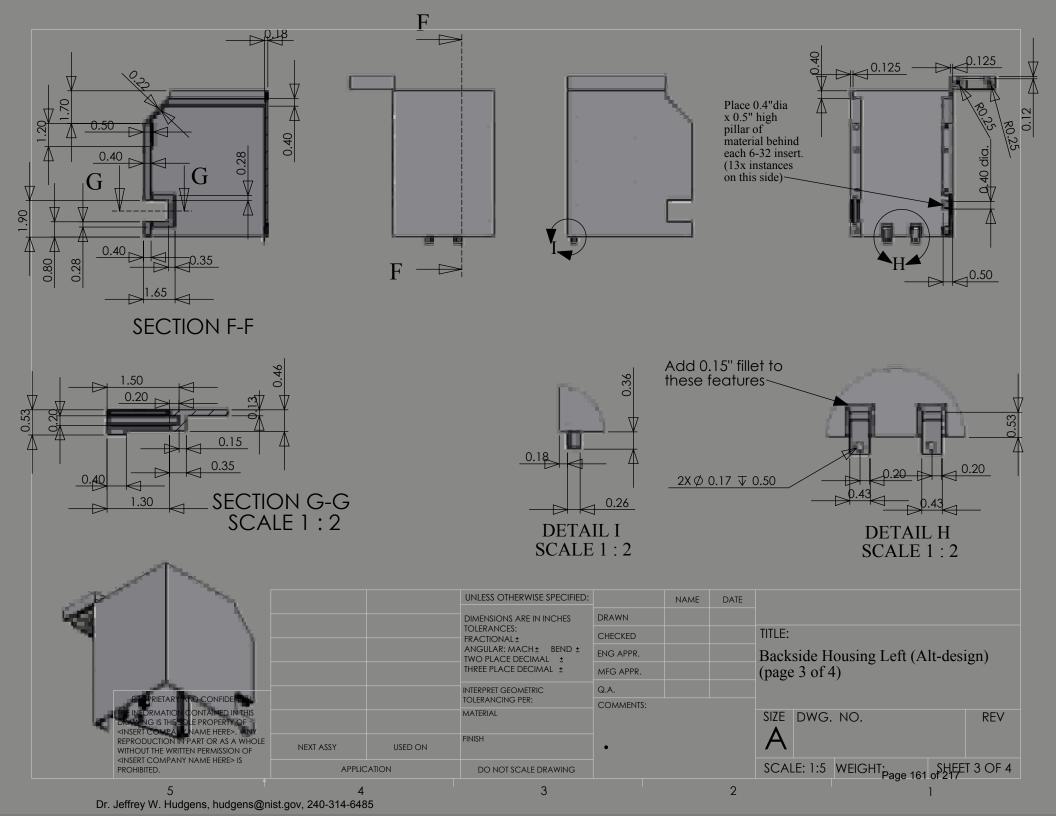


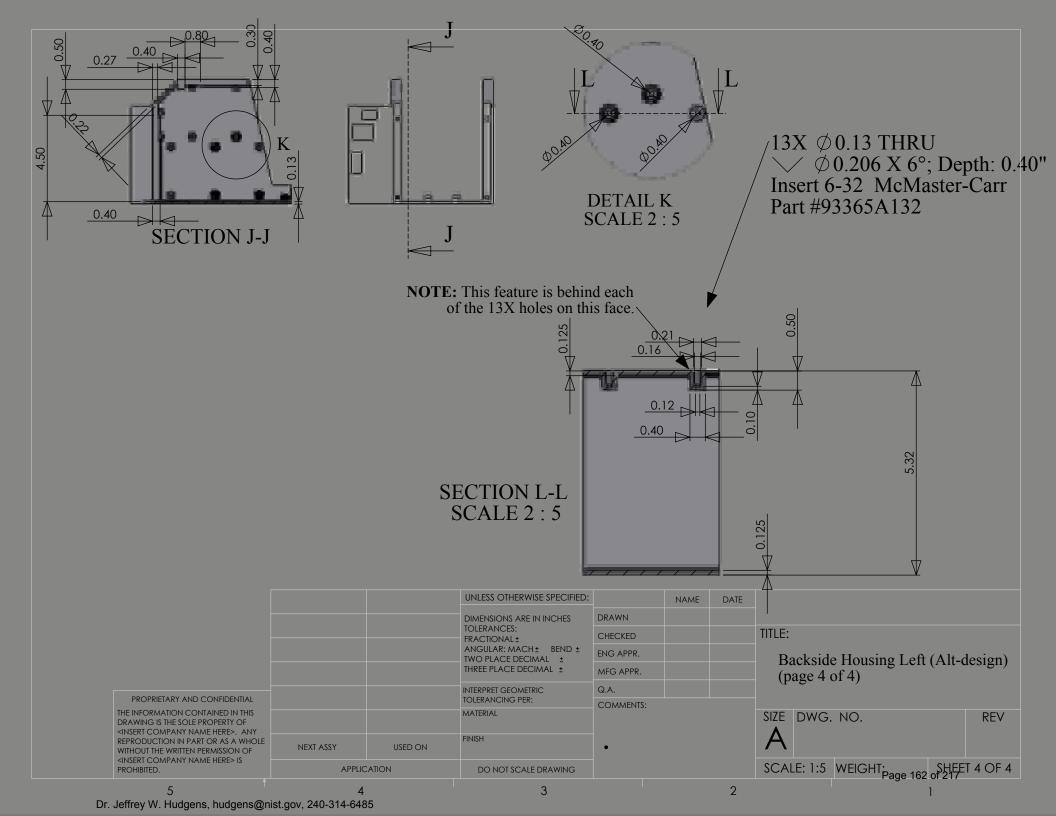


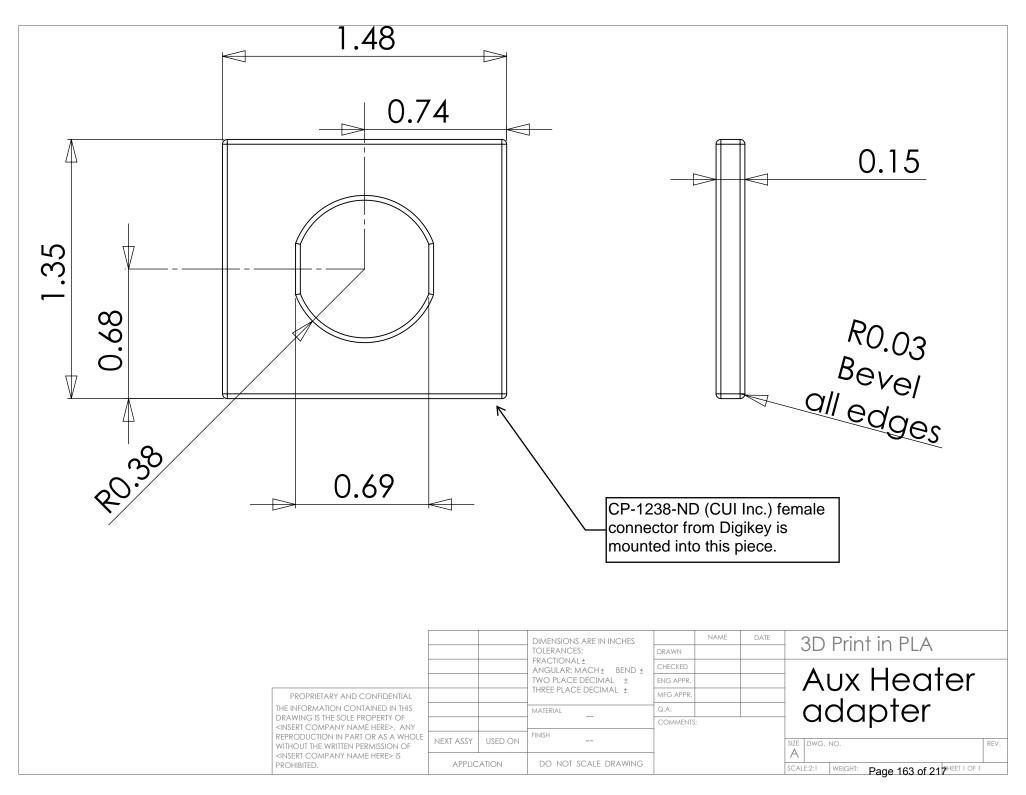
Dr. Jeffrey W. Hudgens, hudgens@nist.gov, 240-314-6485

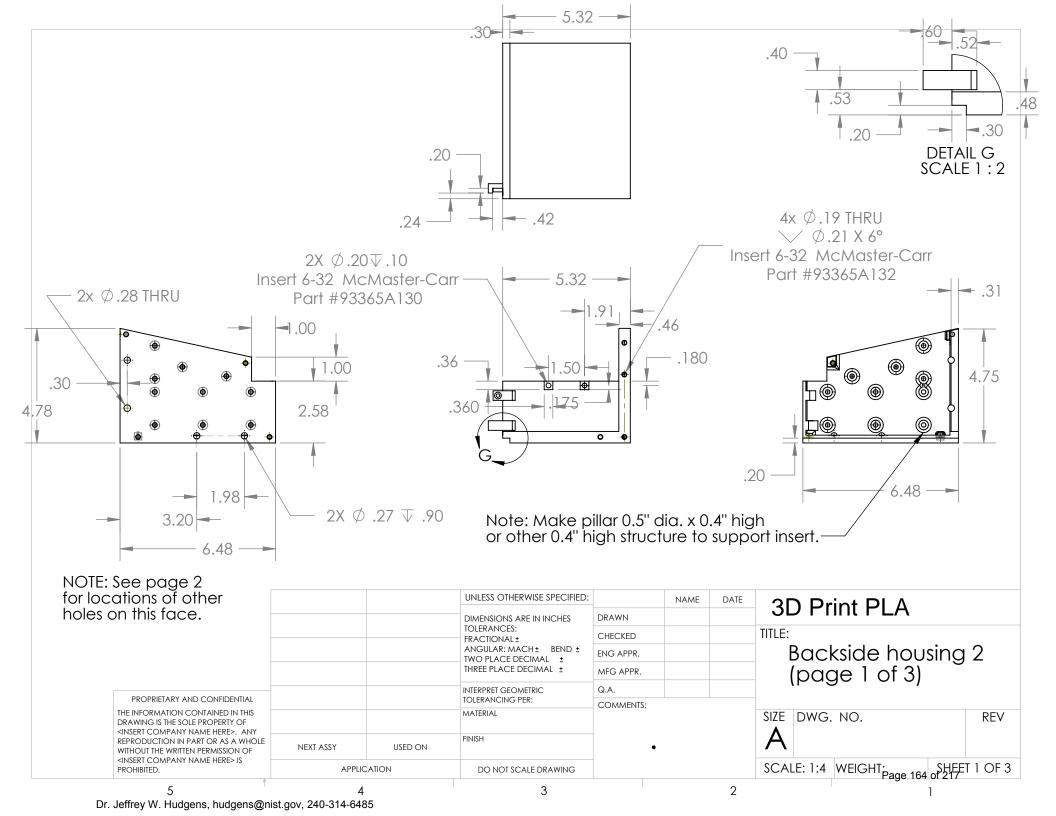


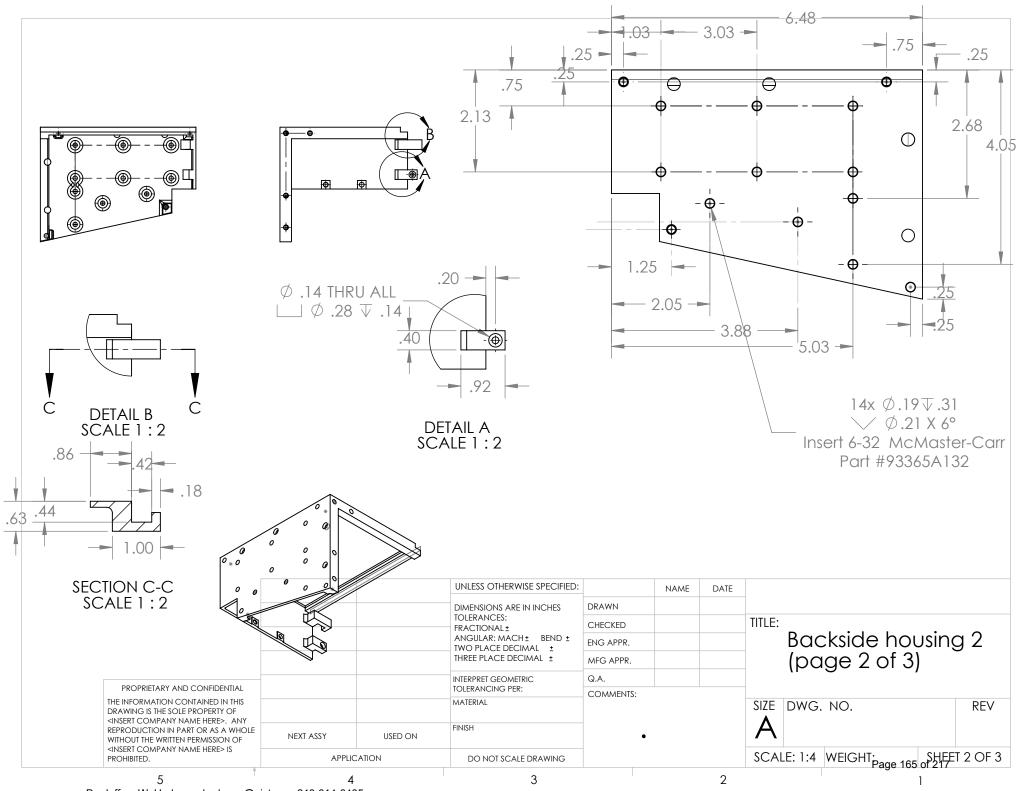


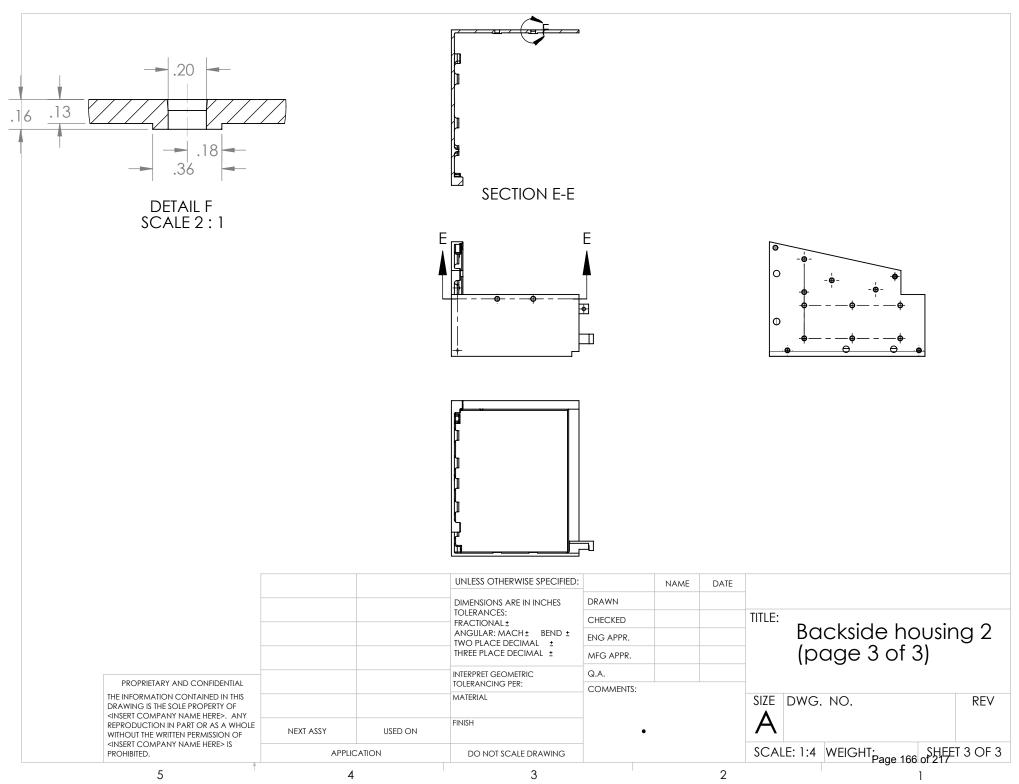


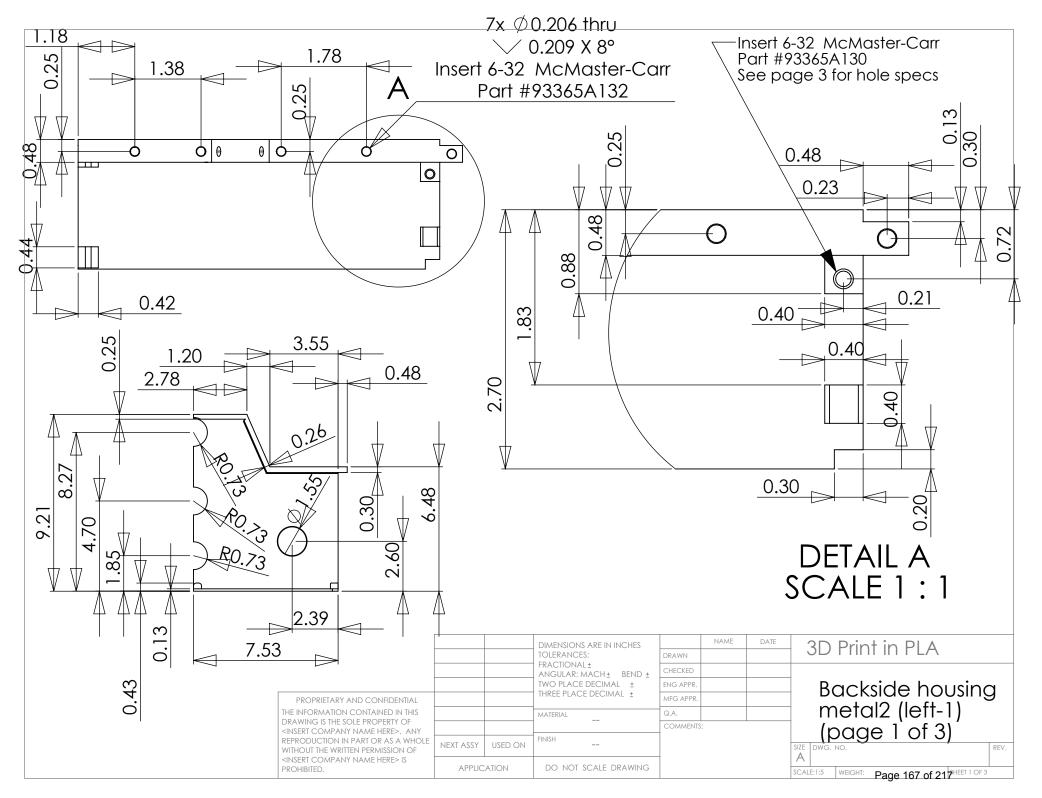


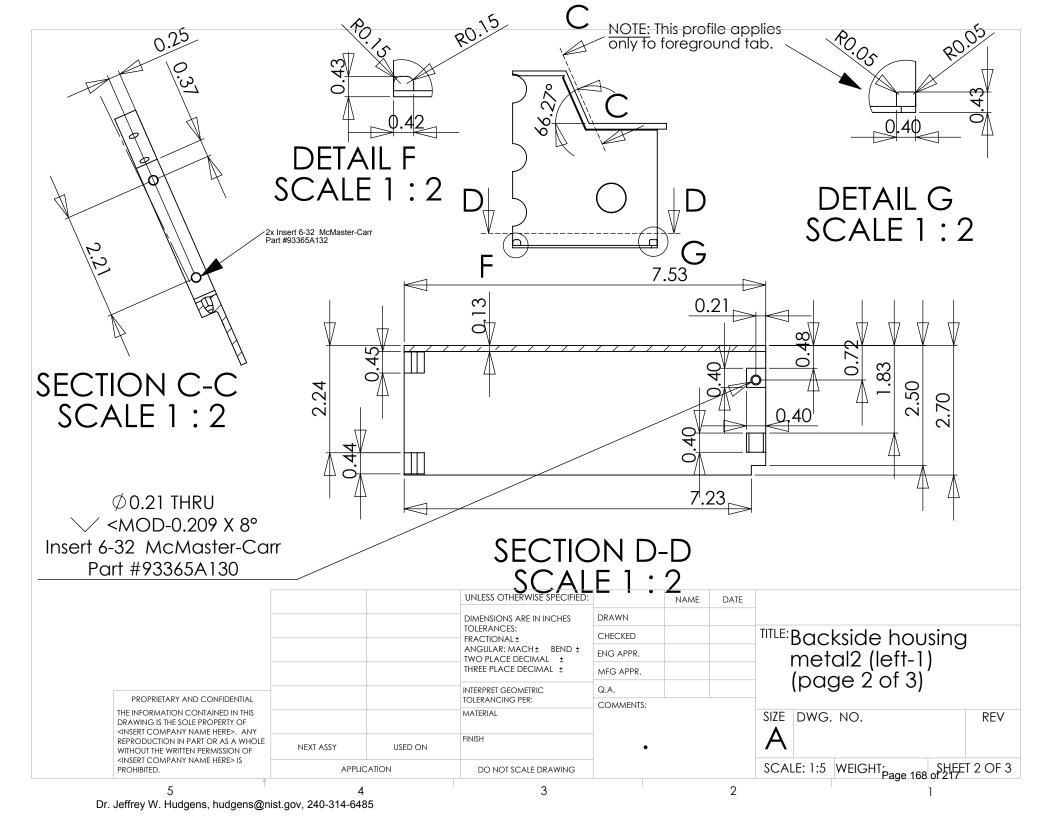


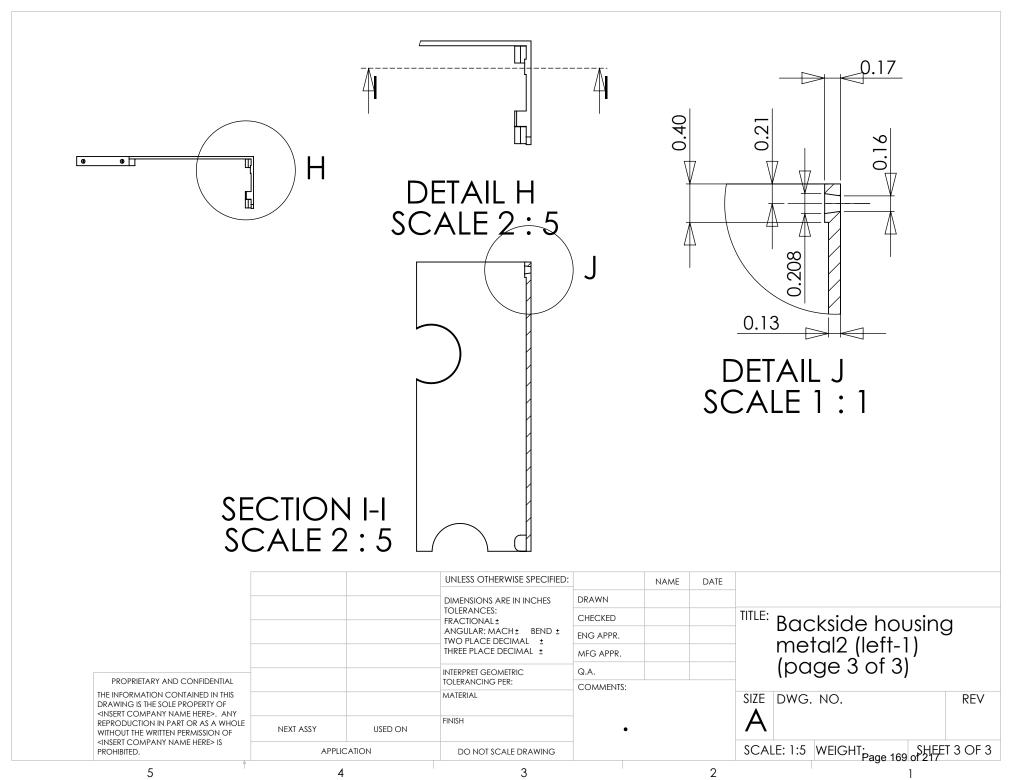


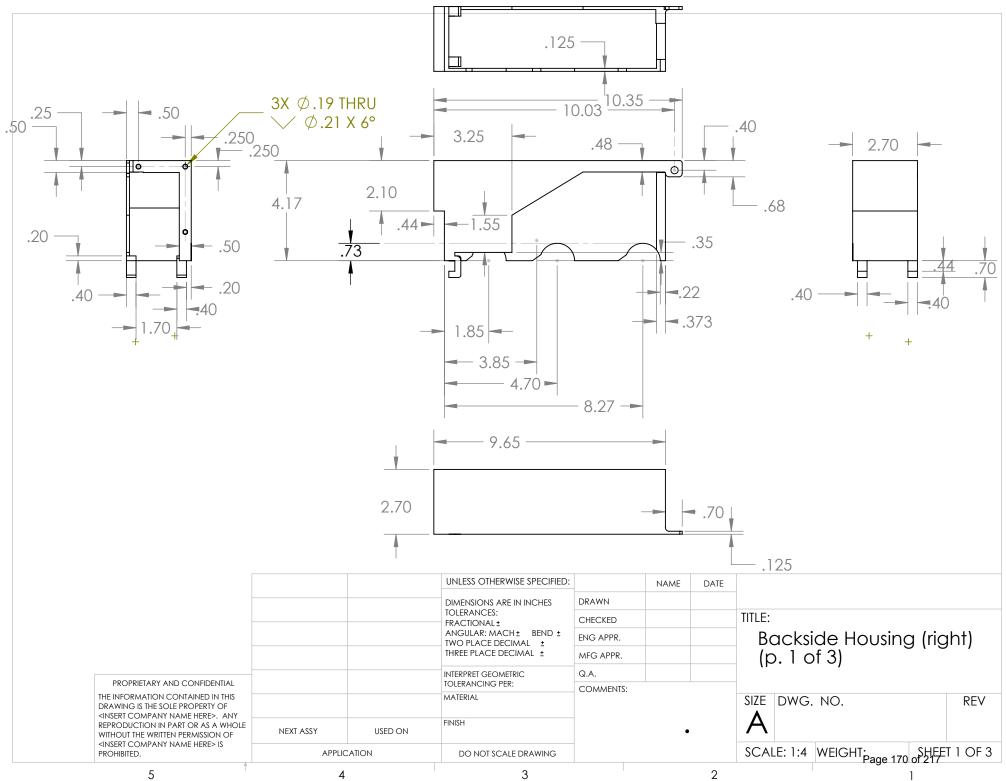


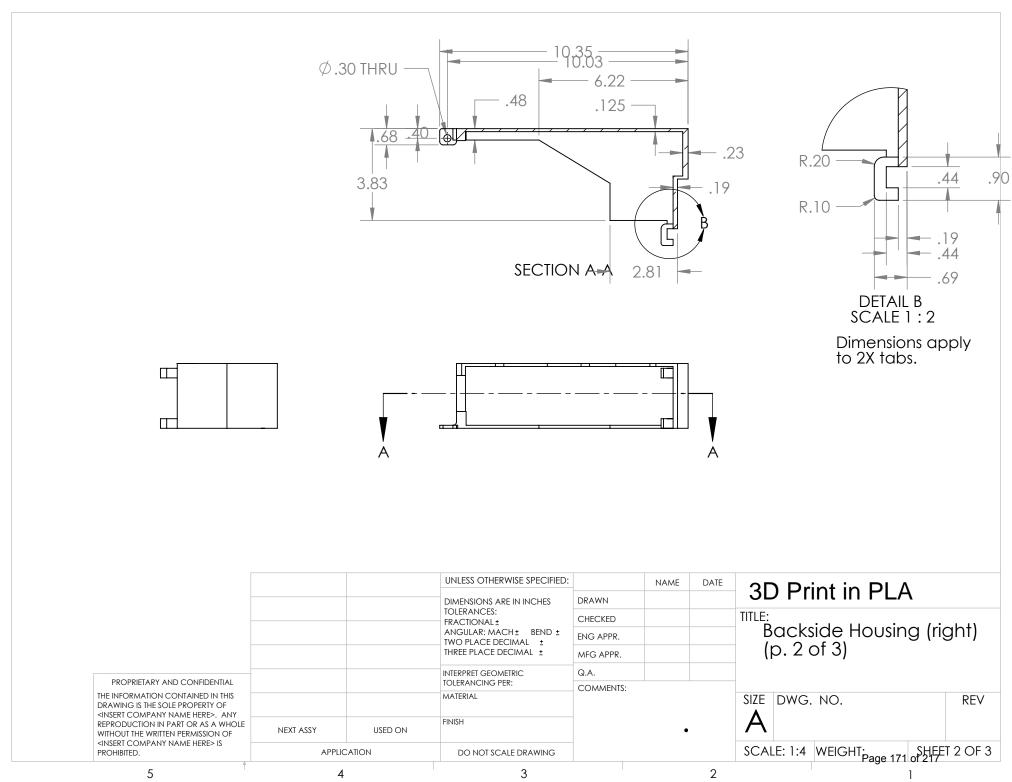


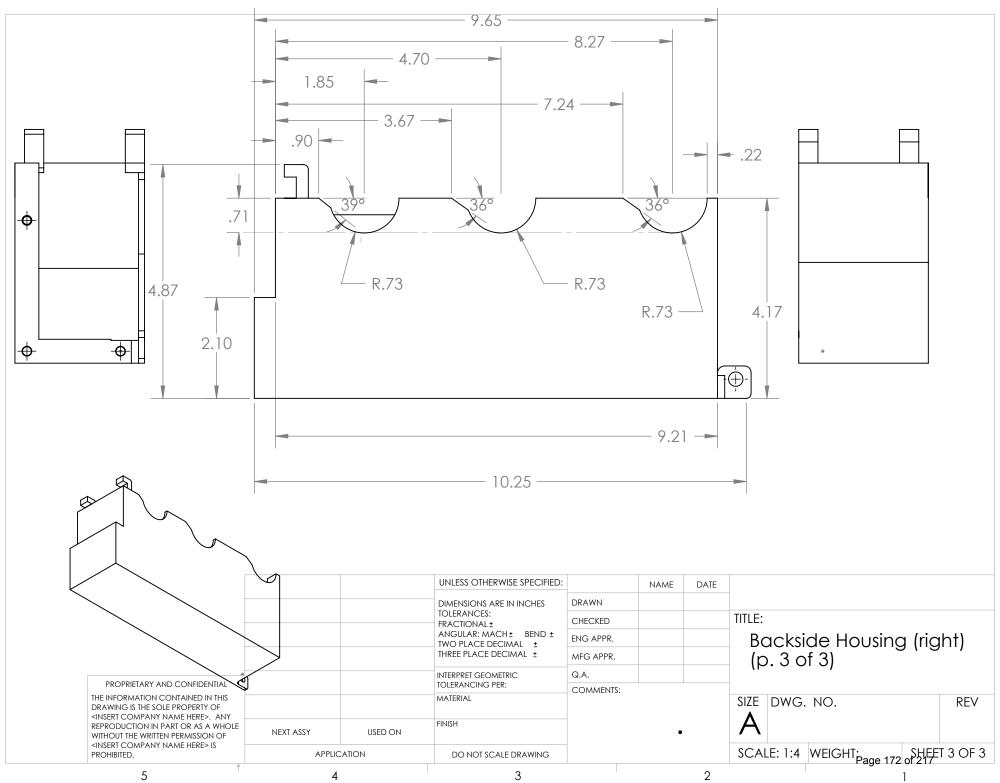


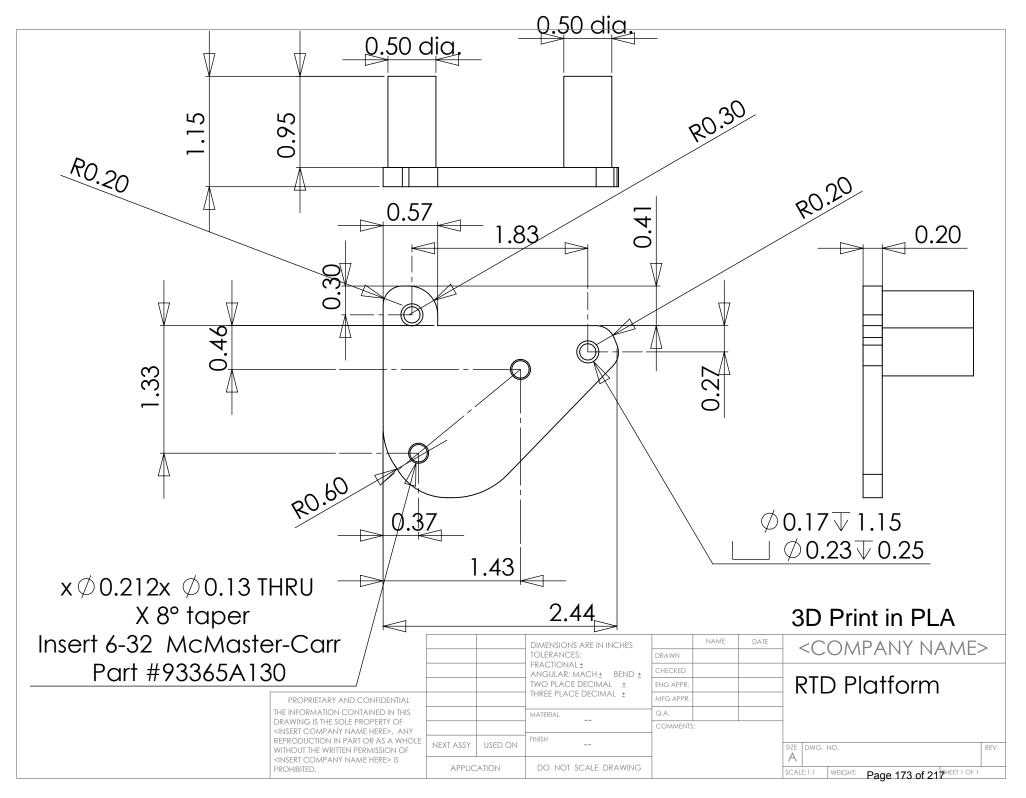


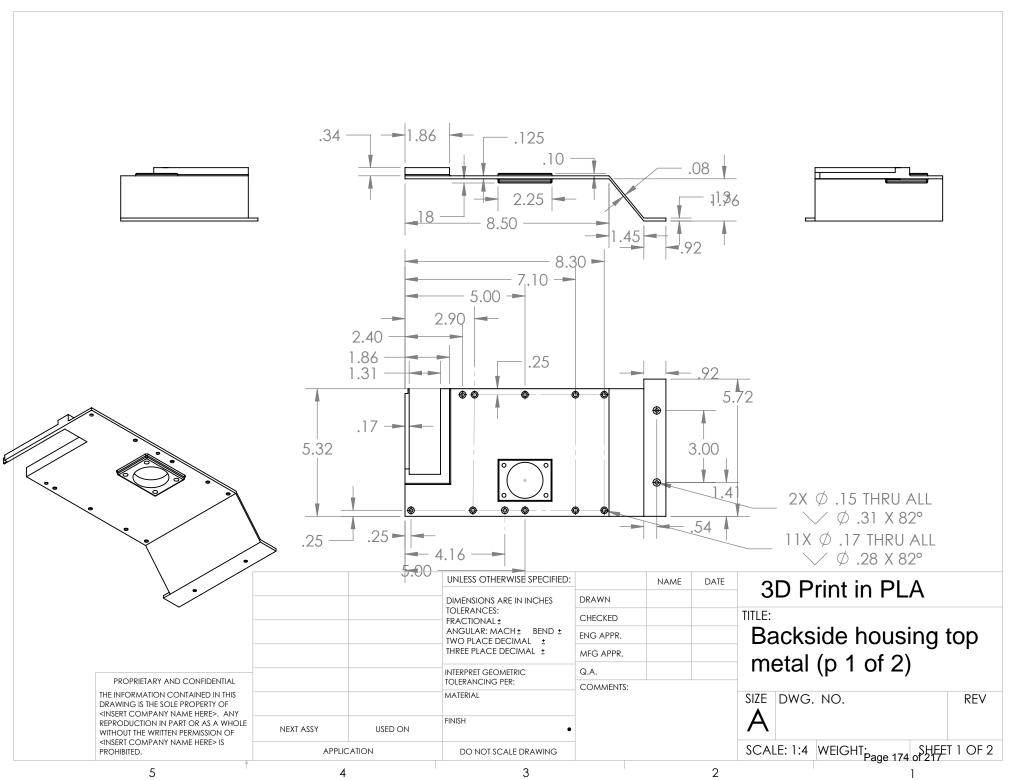


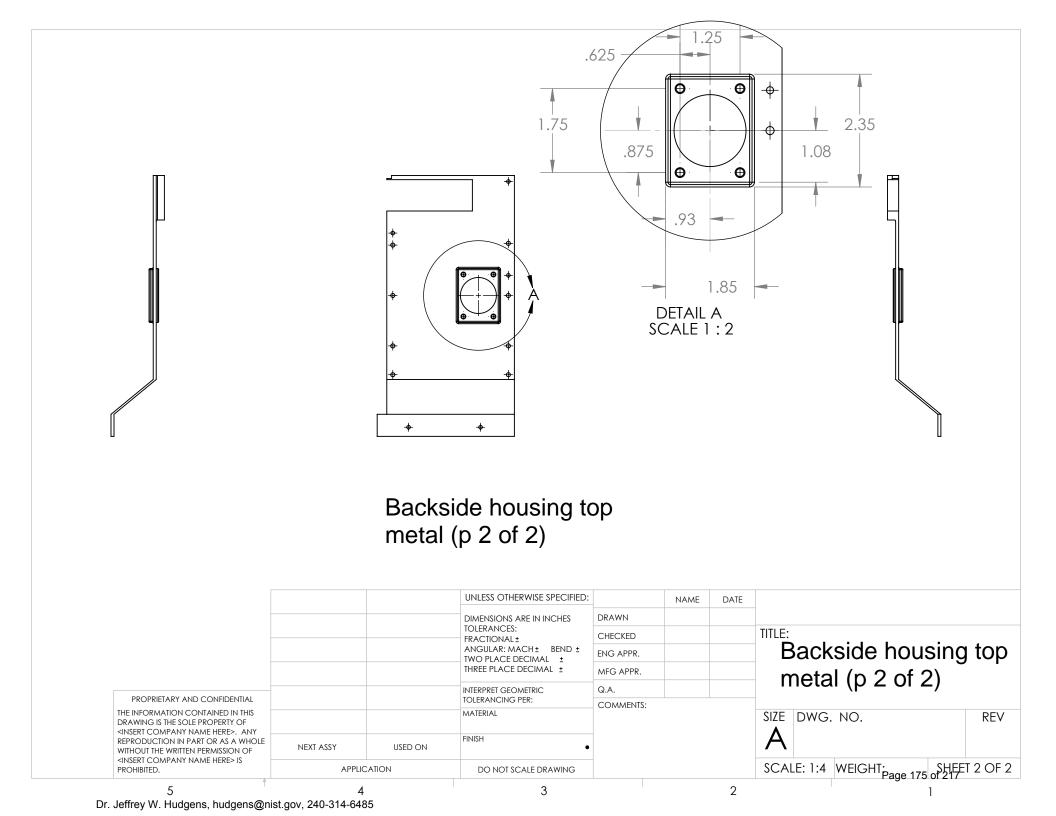


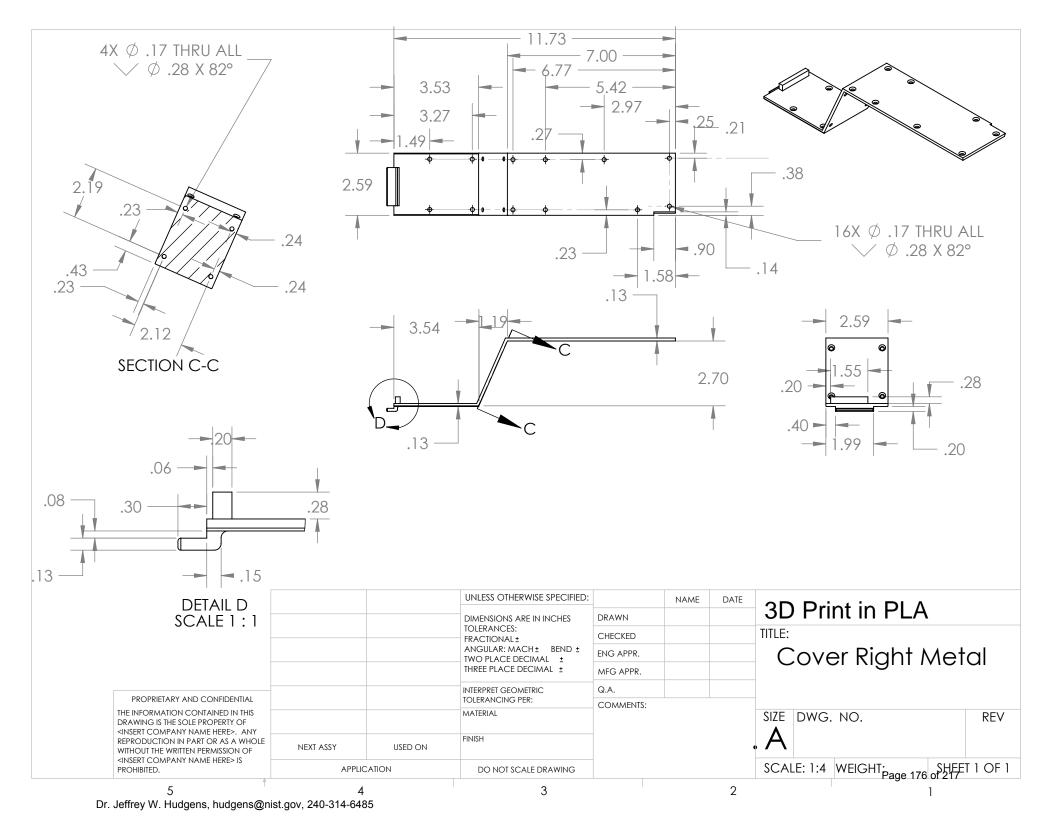


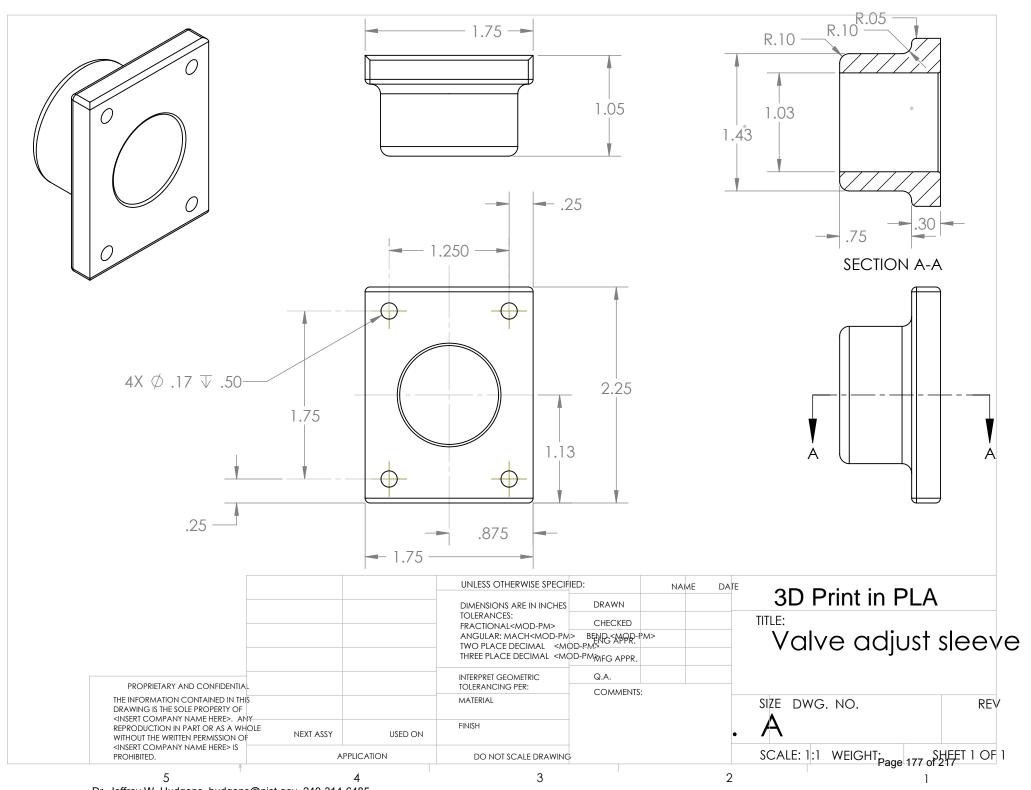


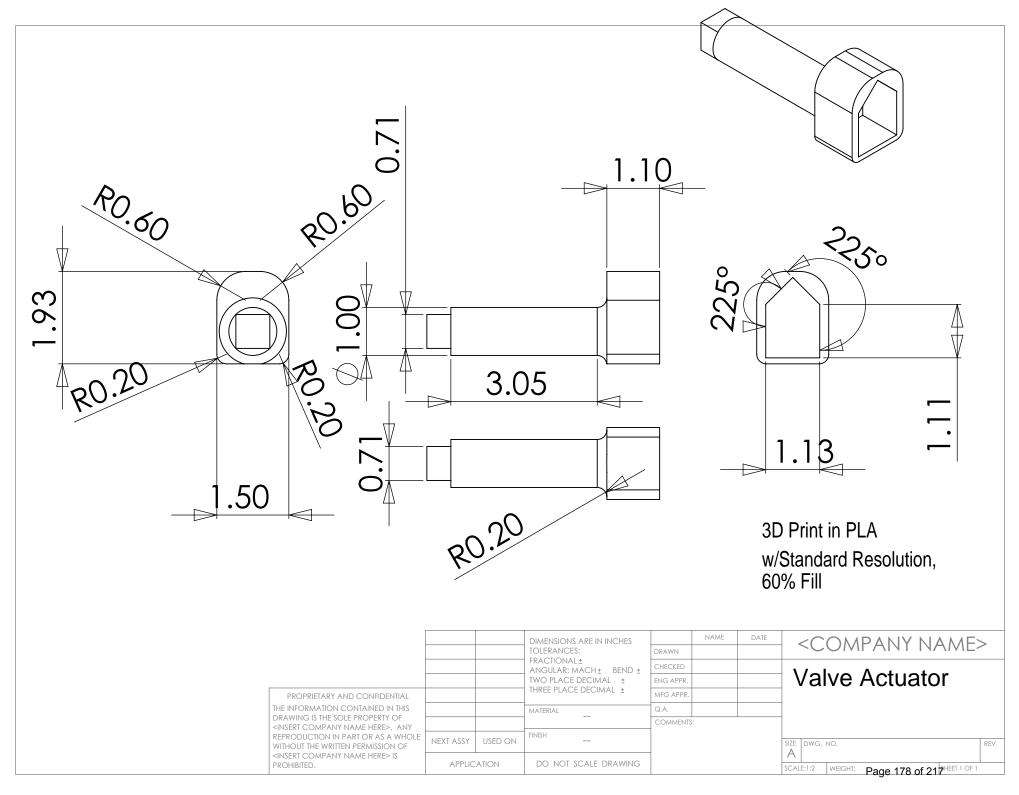


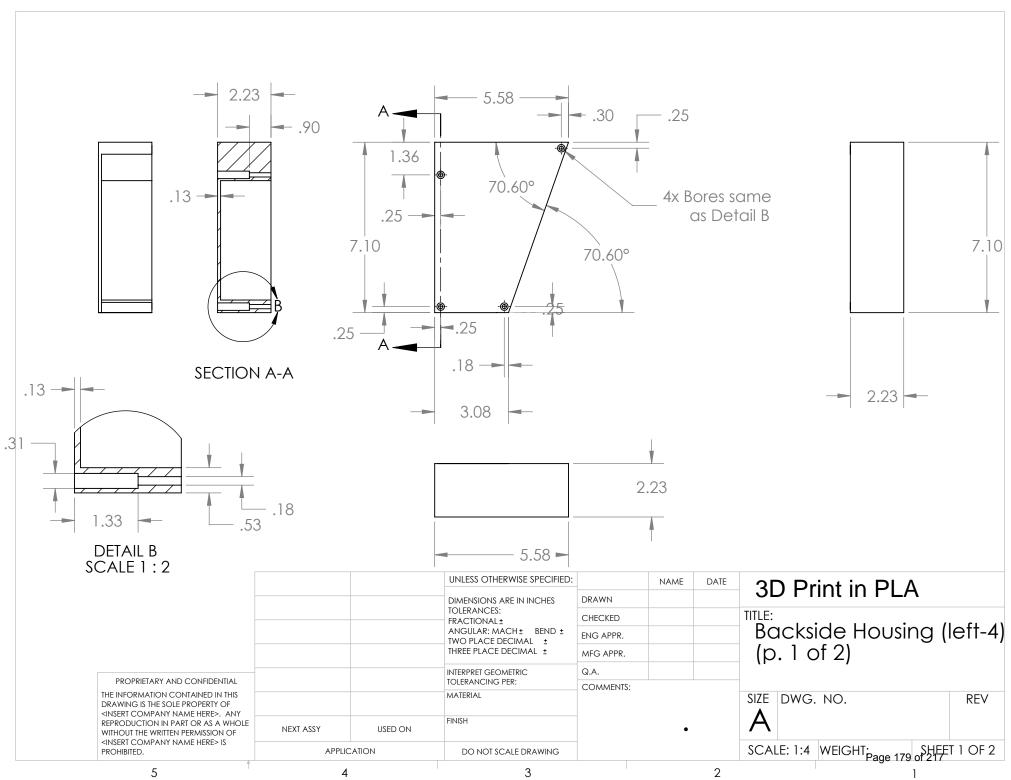


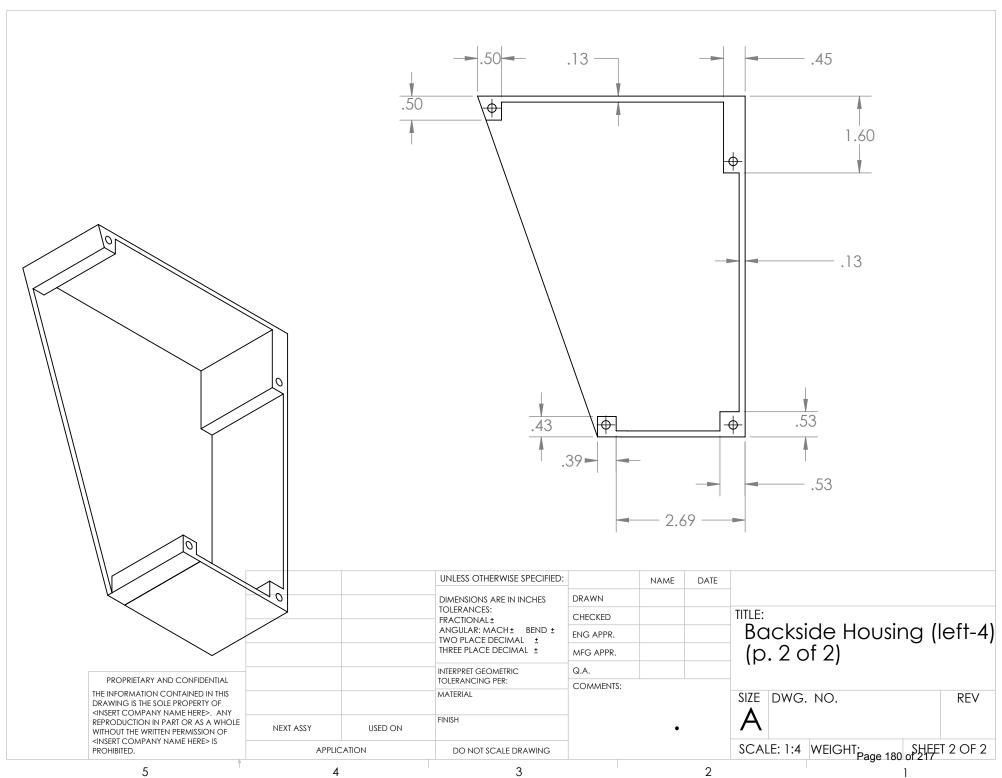


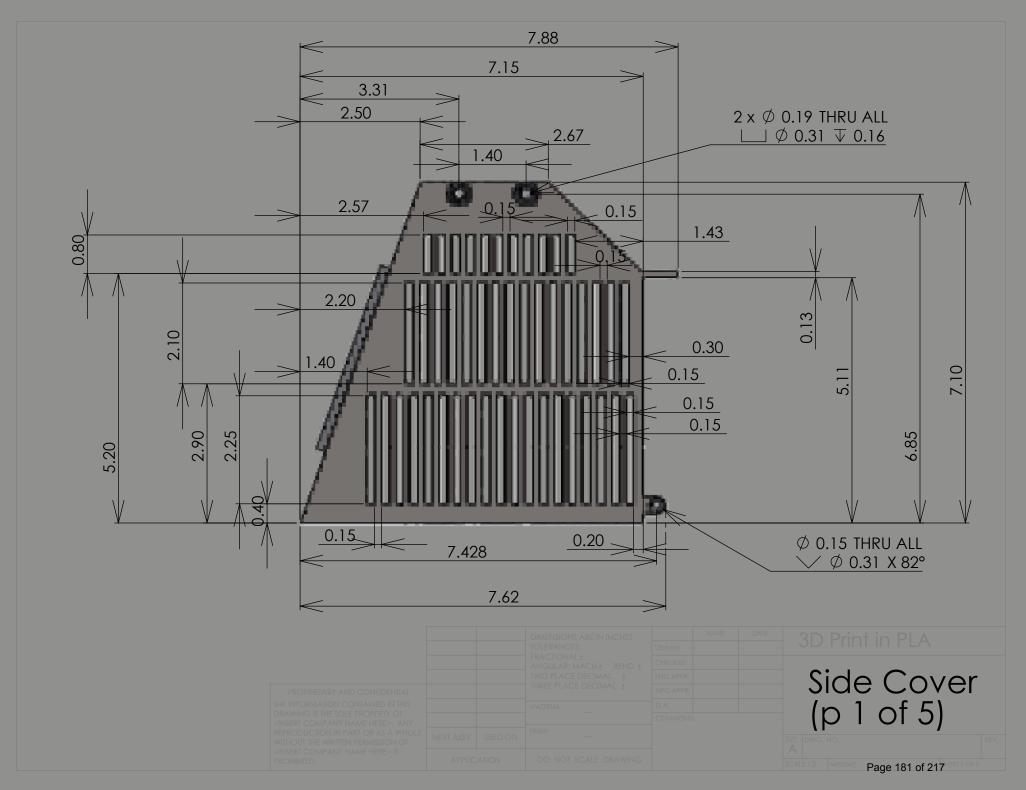


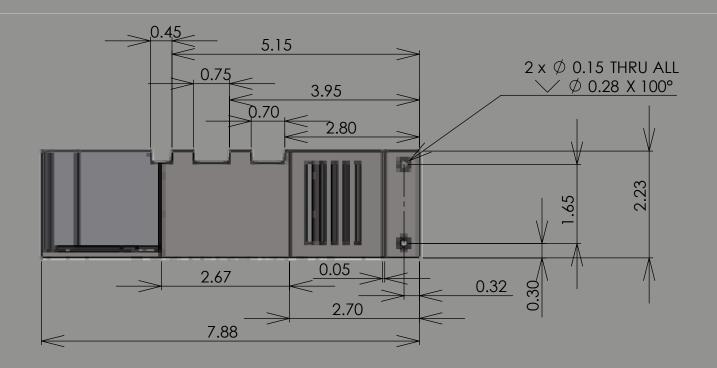


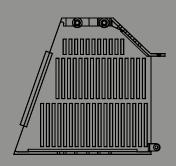








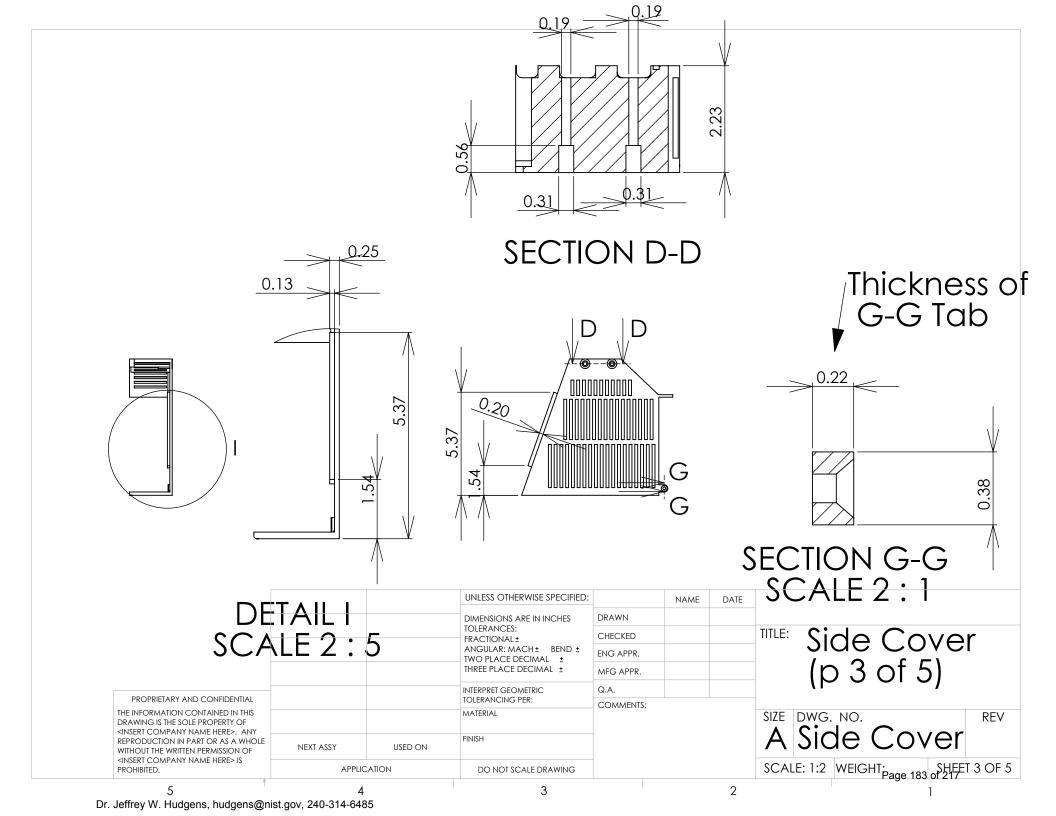


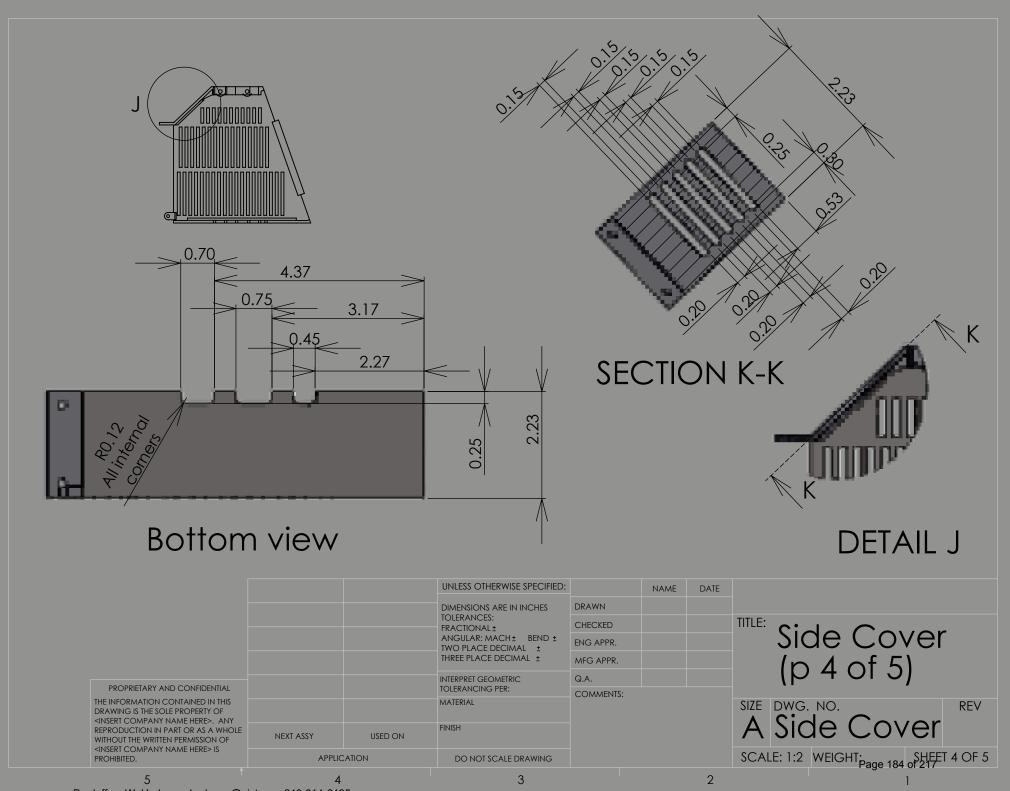


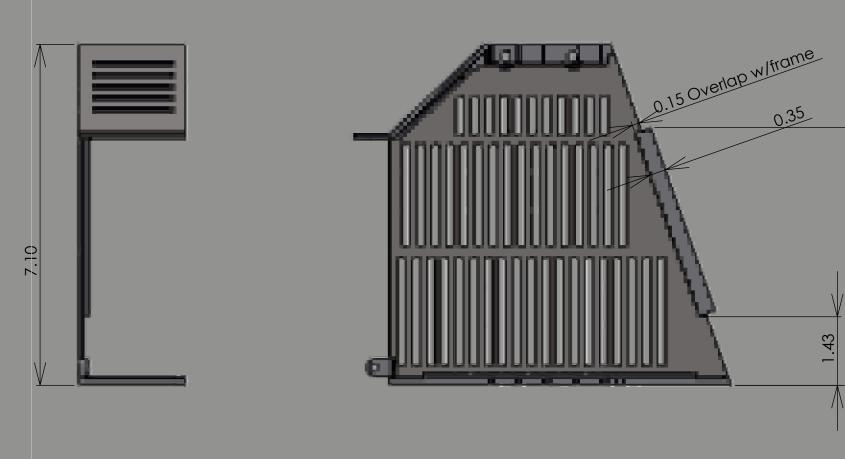
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Side Cover (p 5 of 5)

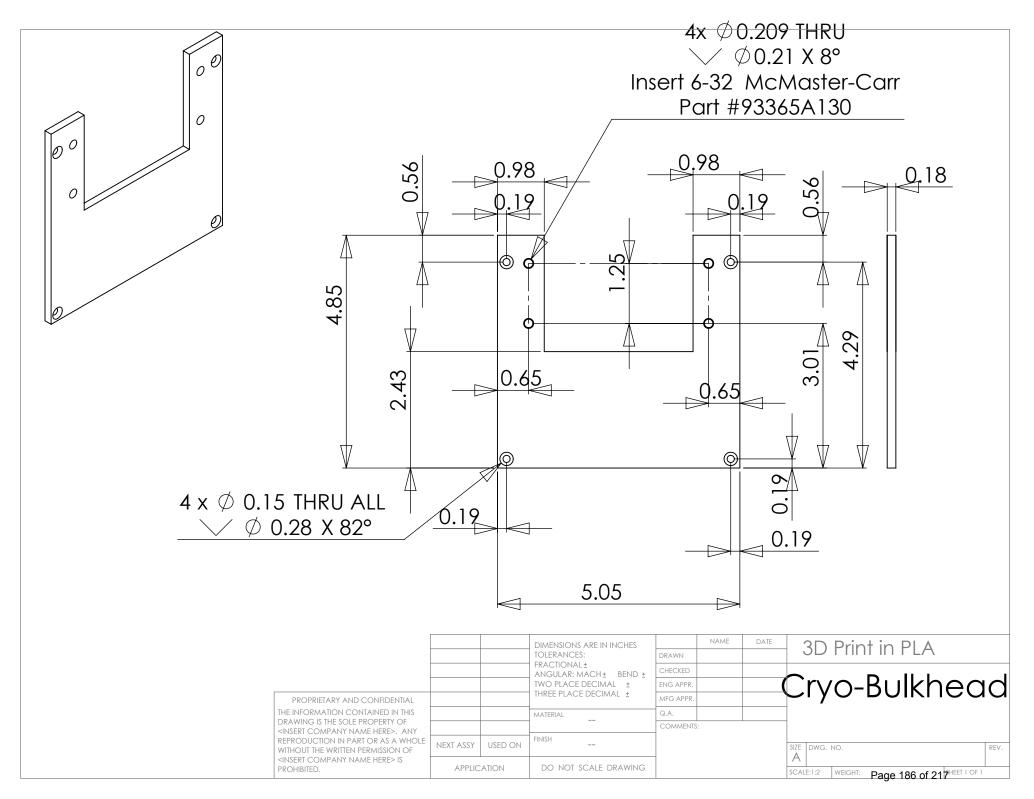
A Side Cover

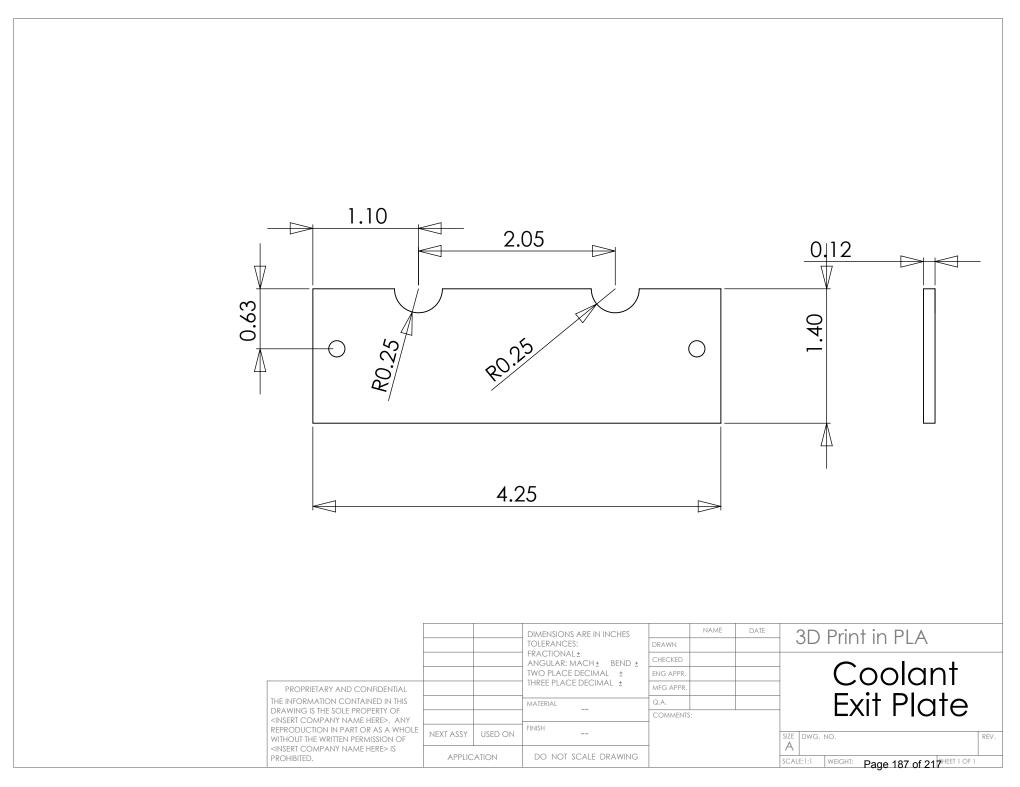
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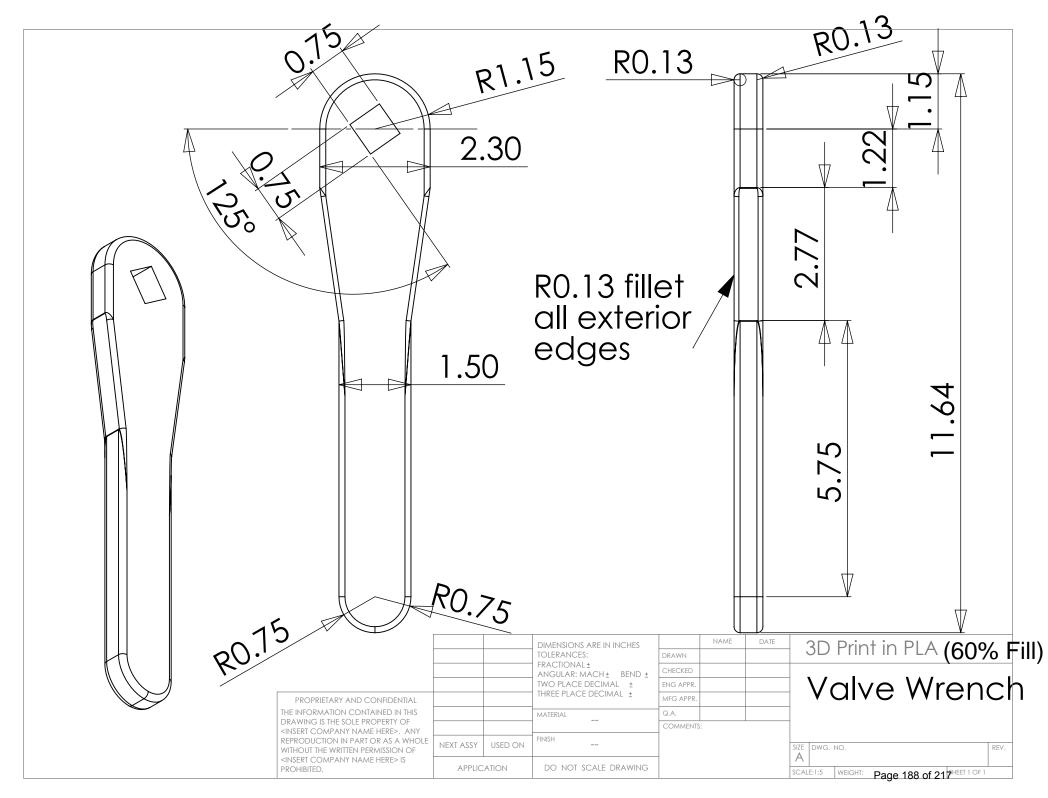
Dr. Jeffrey W. Hudgens, hudgens@nist.gov, 240-314-6485

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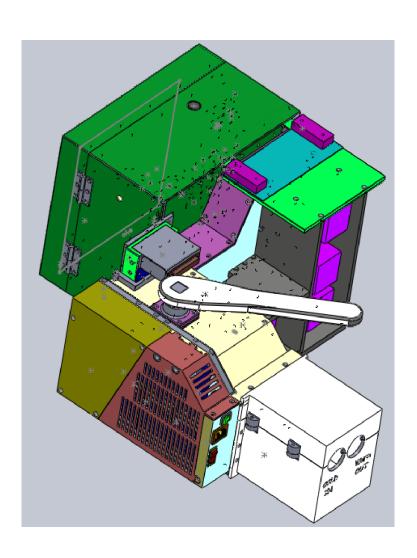
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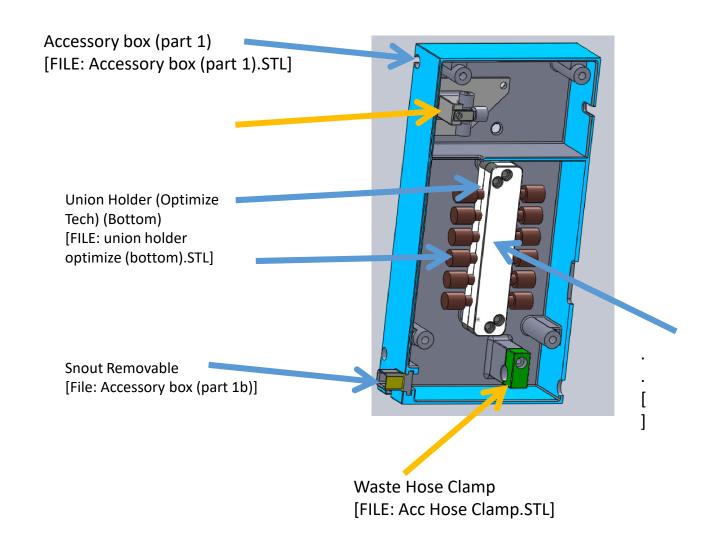


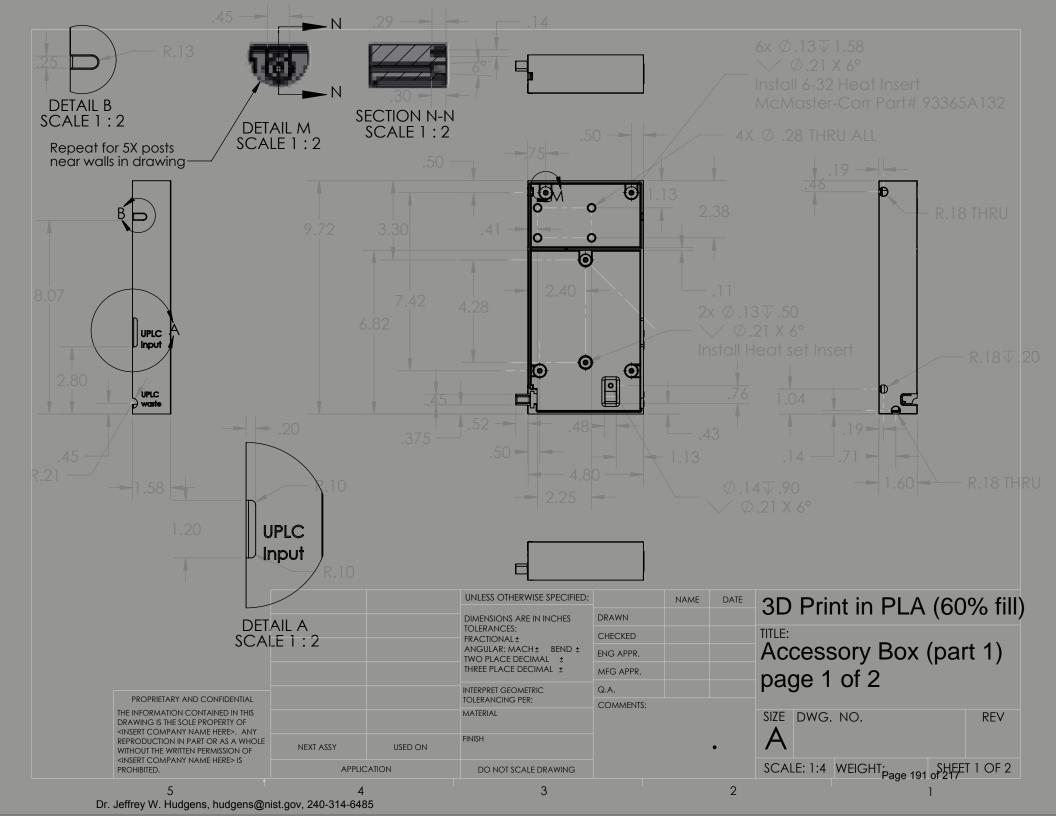


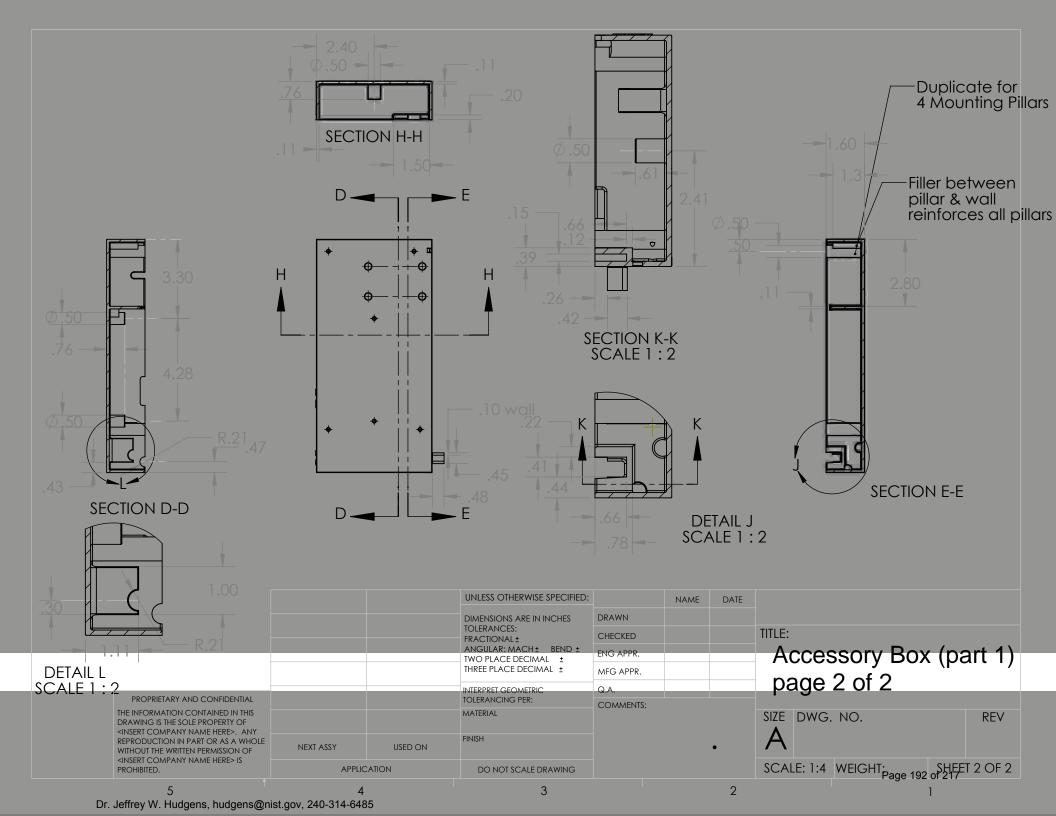
# Showing Assembly of Backside Housing and HPLC

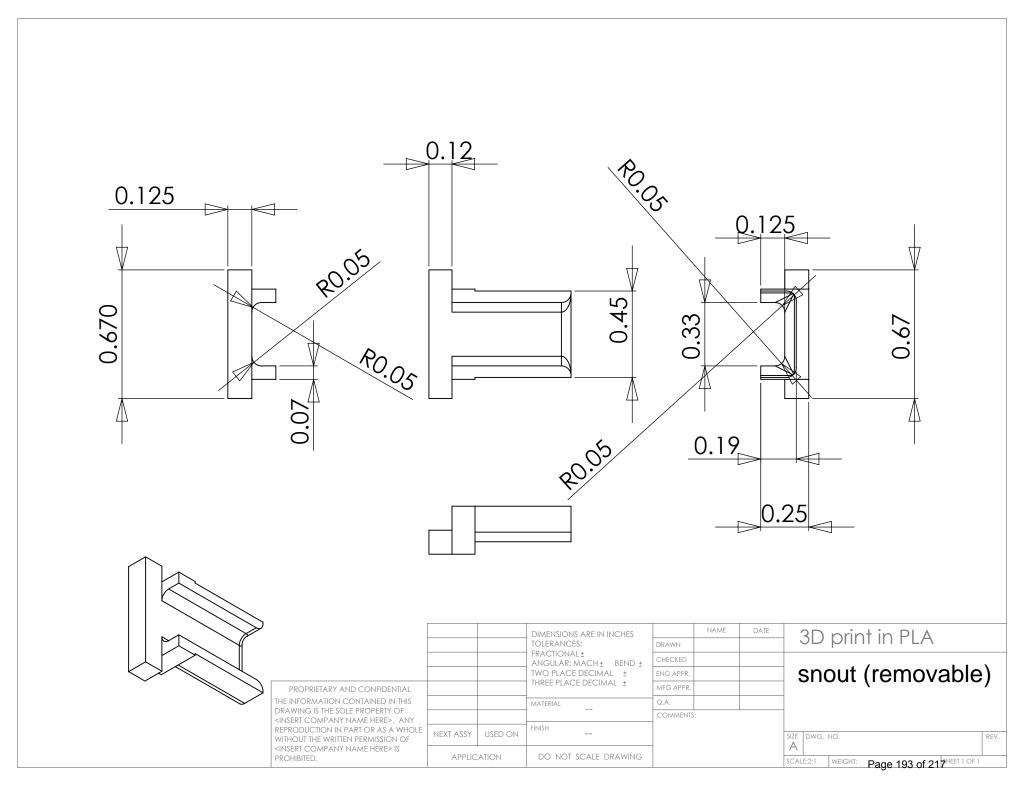


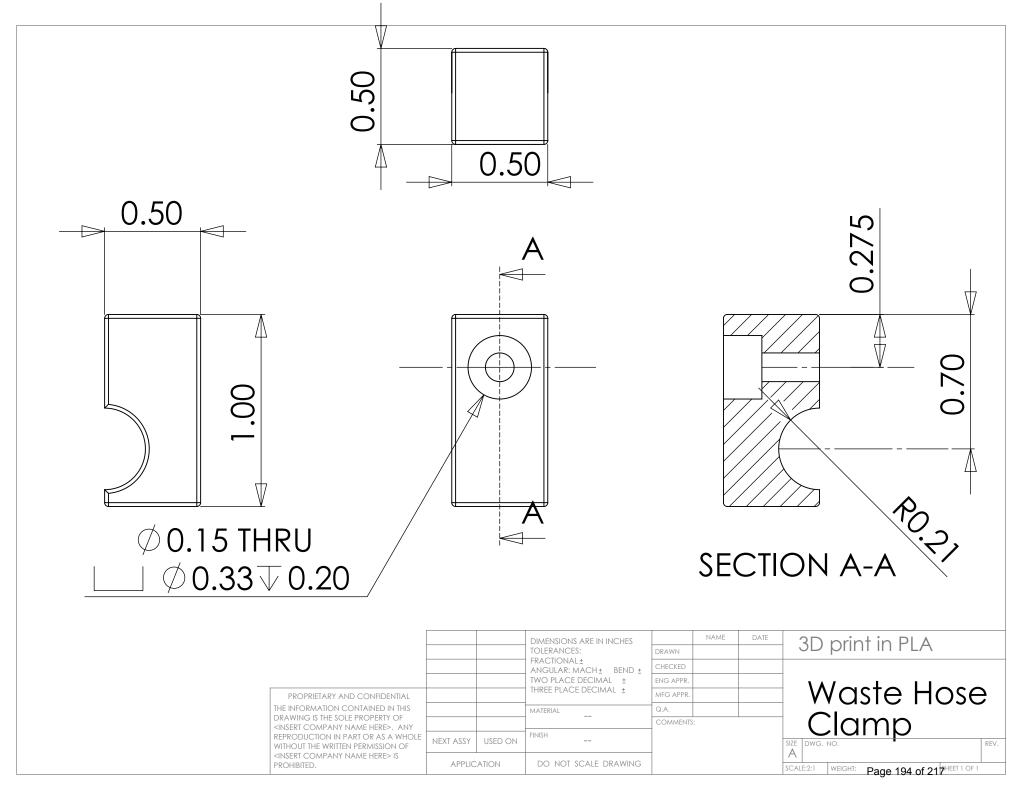
# Accessory Box showing Solvent Entry Rack

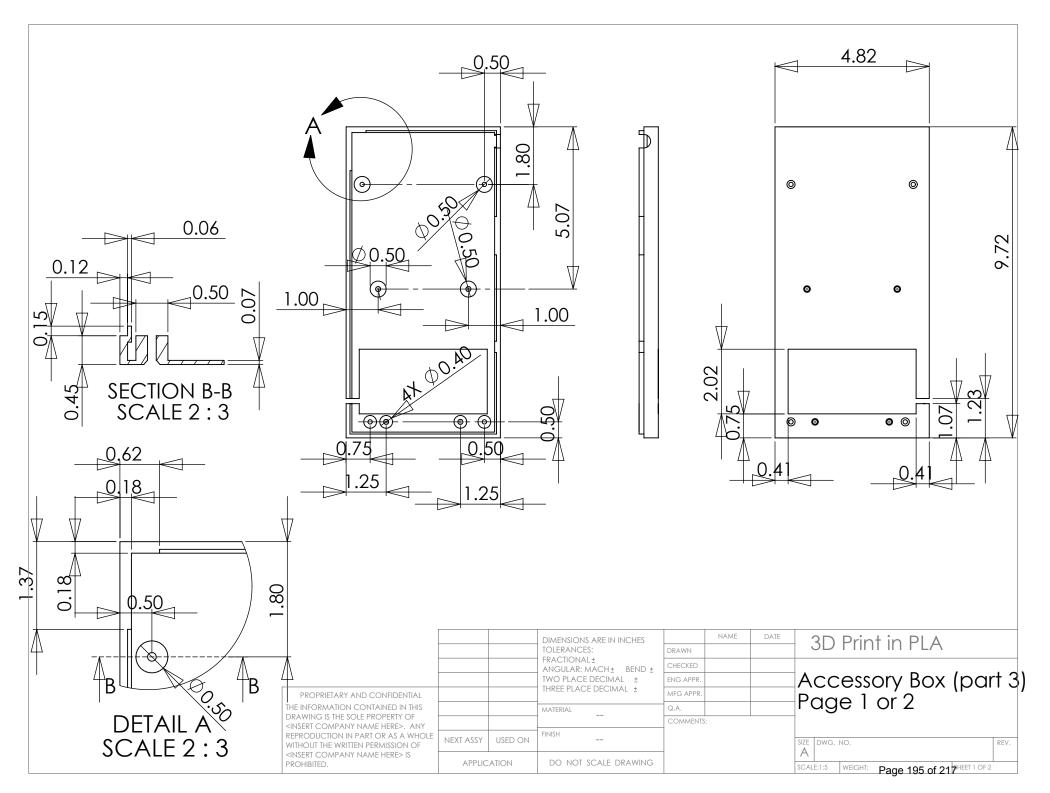


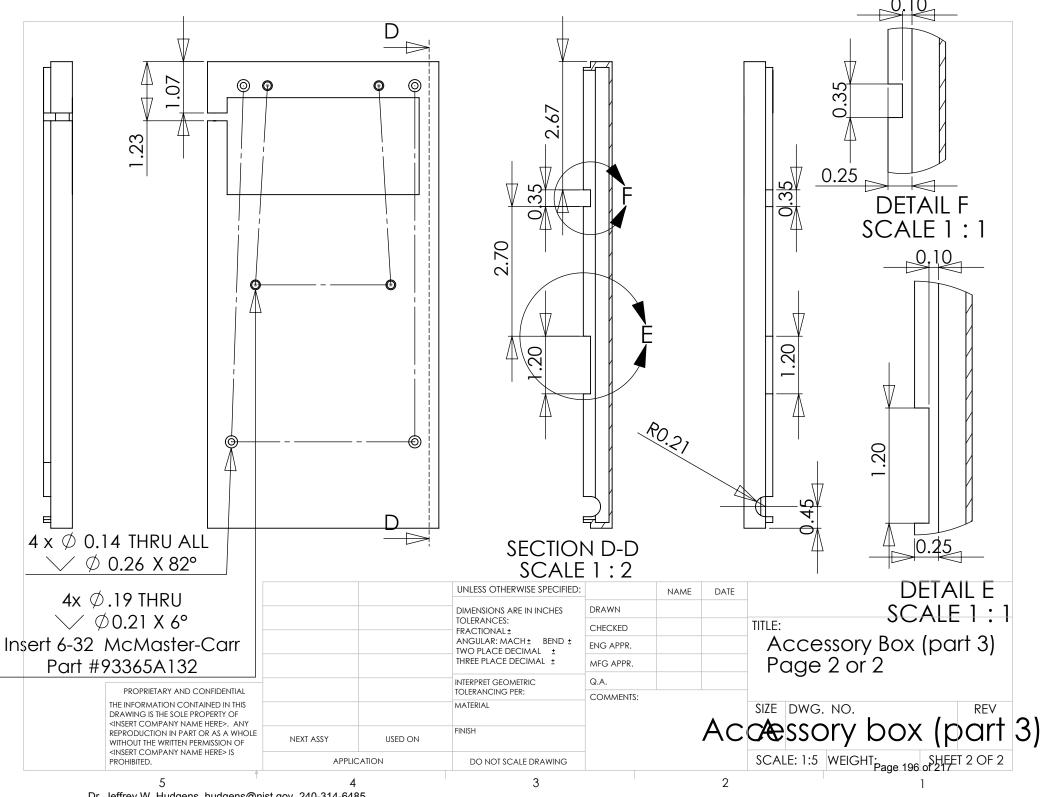


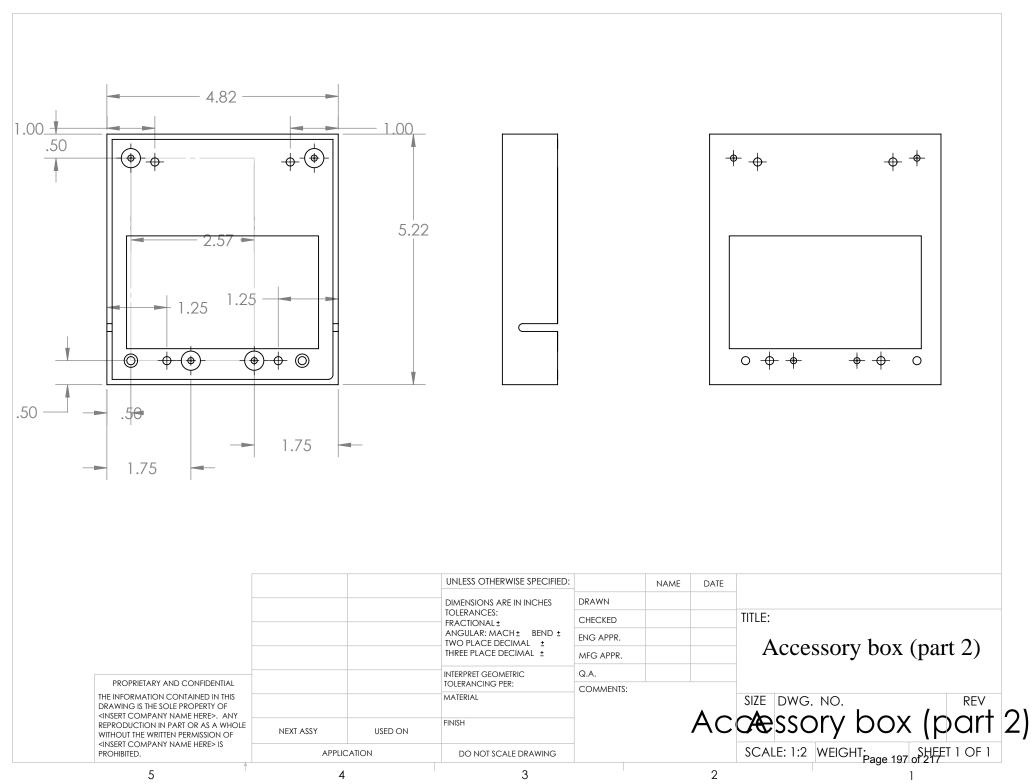




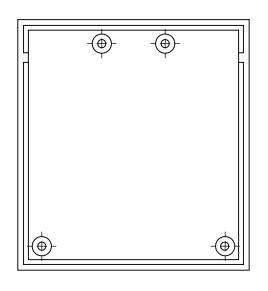


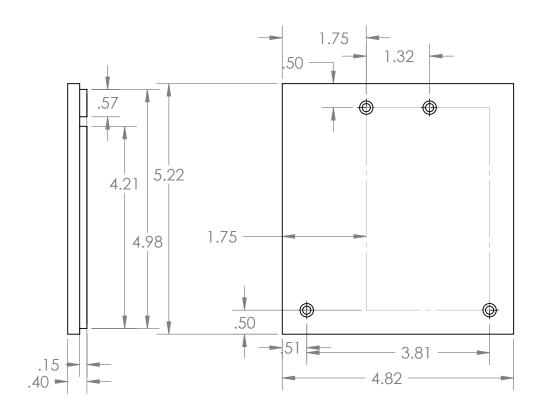






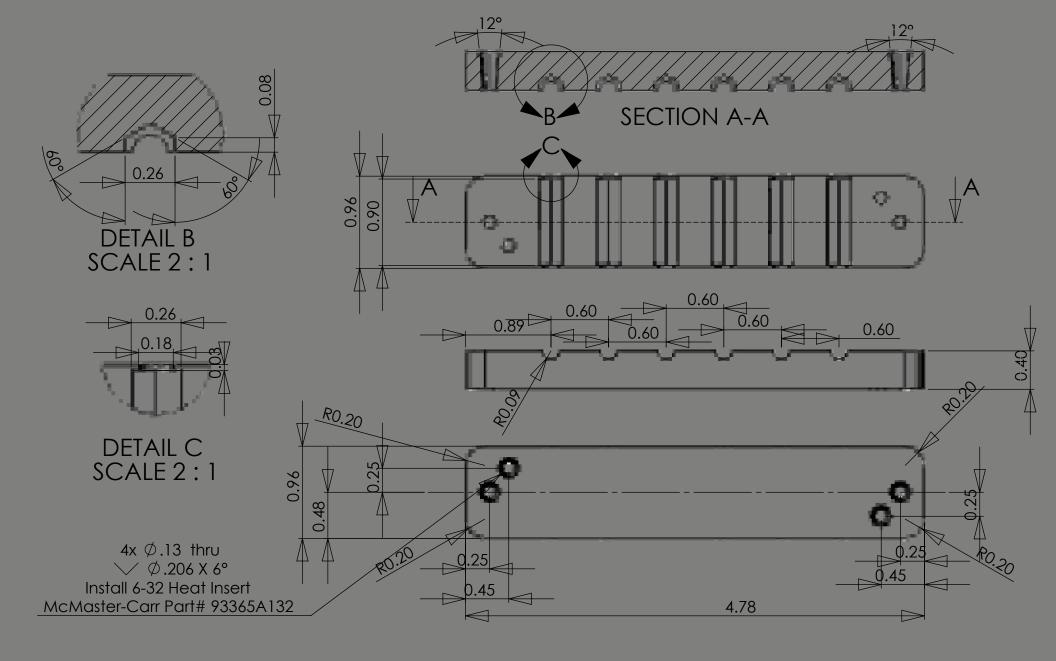
Dr. Jeffrey W. Hudgens, hudgens@nist.gov, 240-314-6485



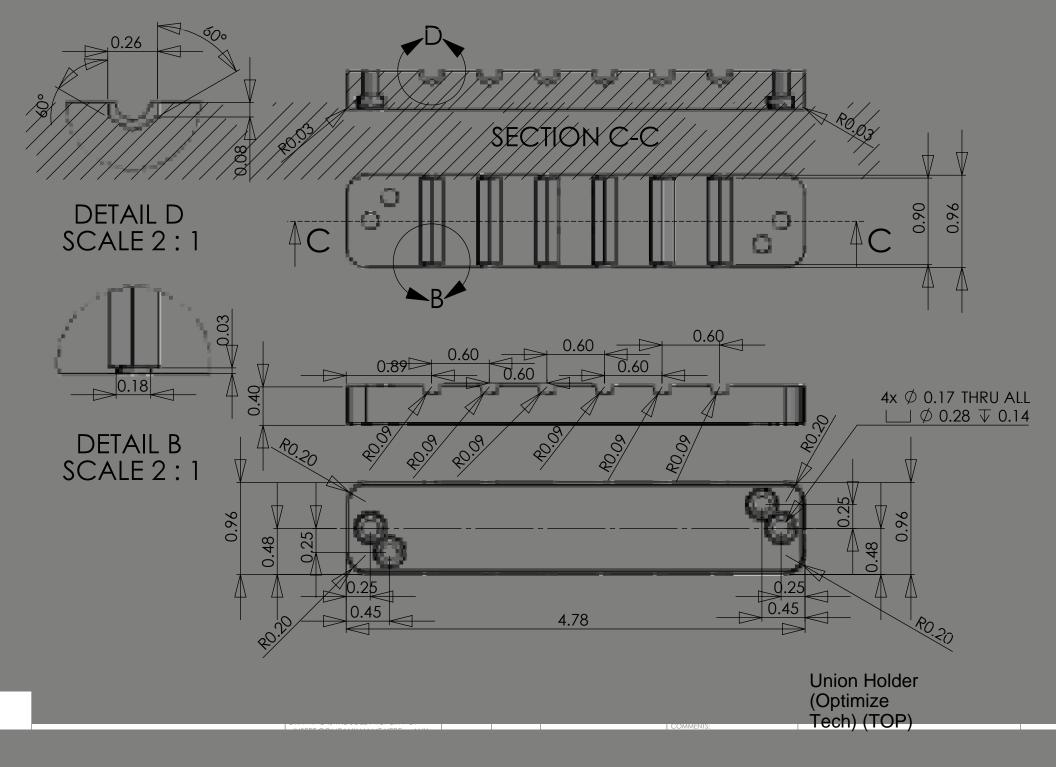


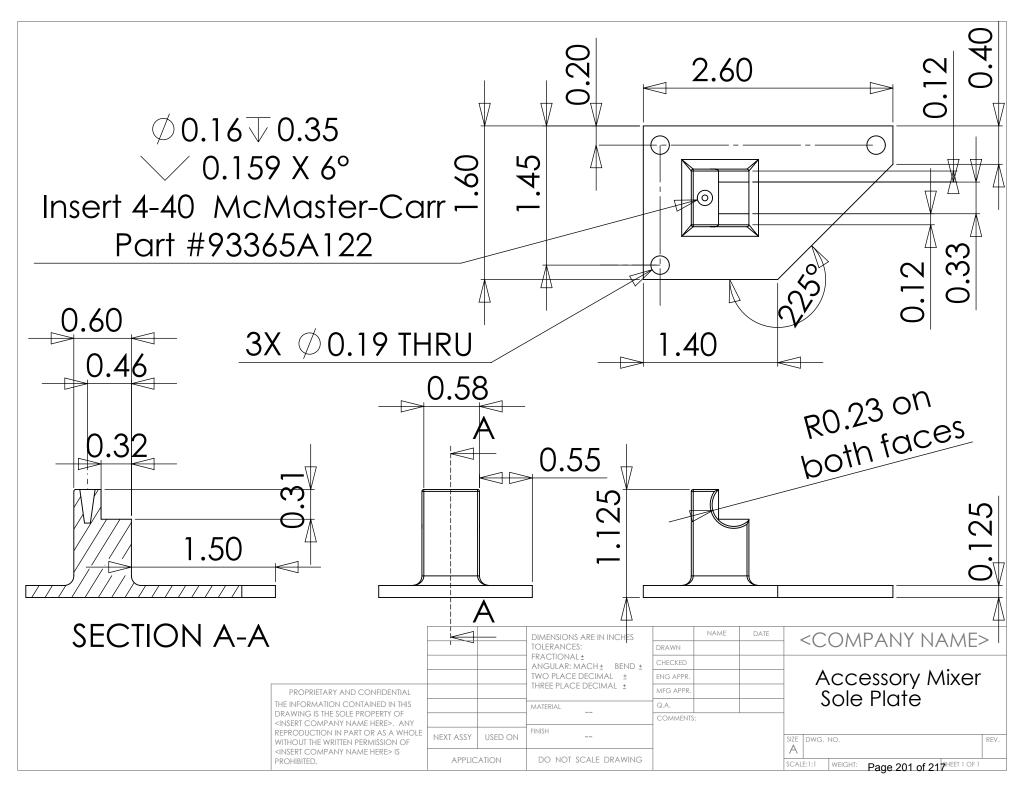
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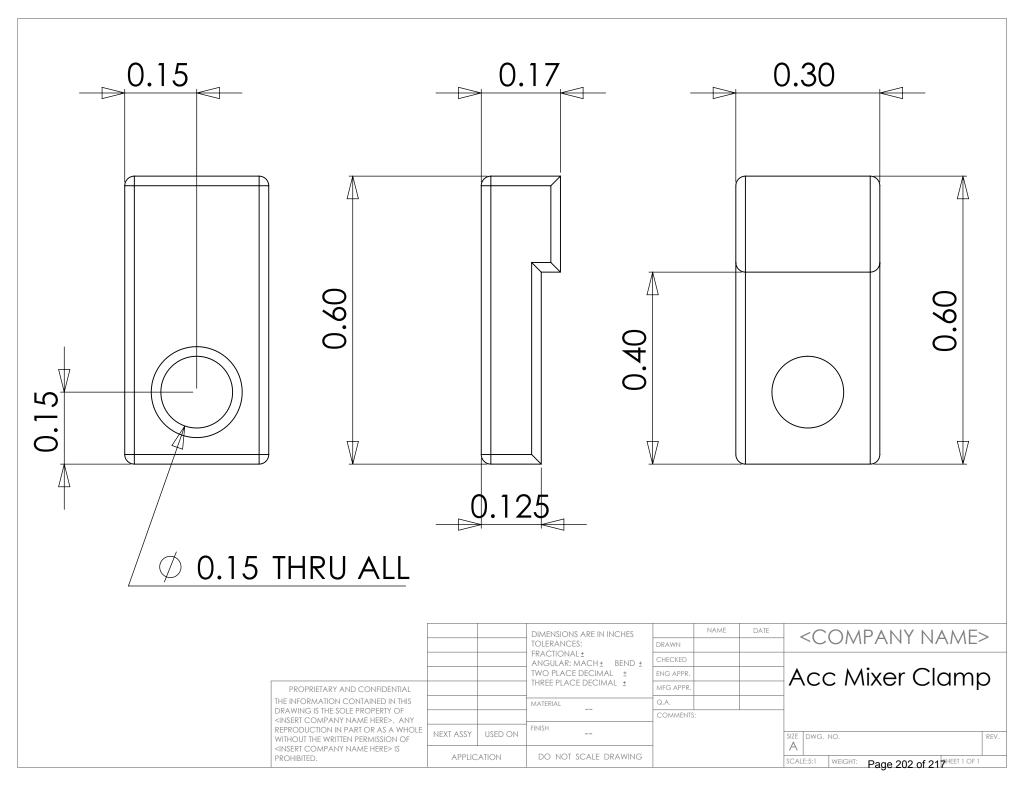
Dr. Jeffrey W. Hudgens, hudgens@nist.gov, 240-314-6485



**Union Holder (Optimize** Tech) (Bottom)







## View of Backside Electrical Connections

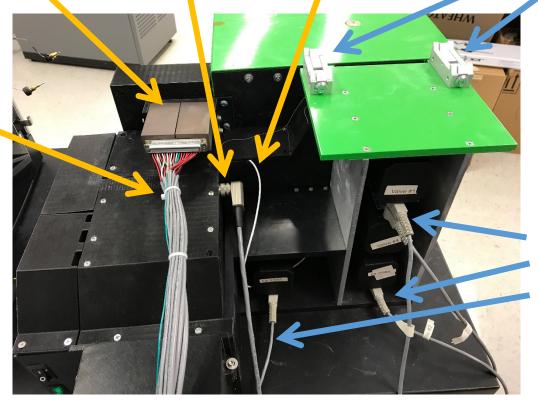
-- View of an older prototype housing. The connections are same as in the newer housing given in this document.

37-pin Sensor-Power Entry Connector (SPEC)

9-pin Sensor-Power Connector for heater elements on each 6" valve driver shaft. Cable connects to Temperature Controller Cable to thermistor that senses temperature on Valve #3 driver shaft & 37-pin SPEC

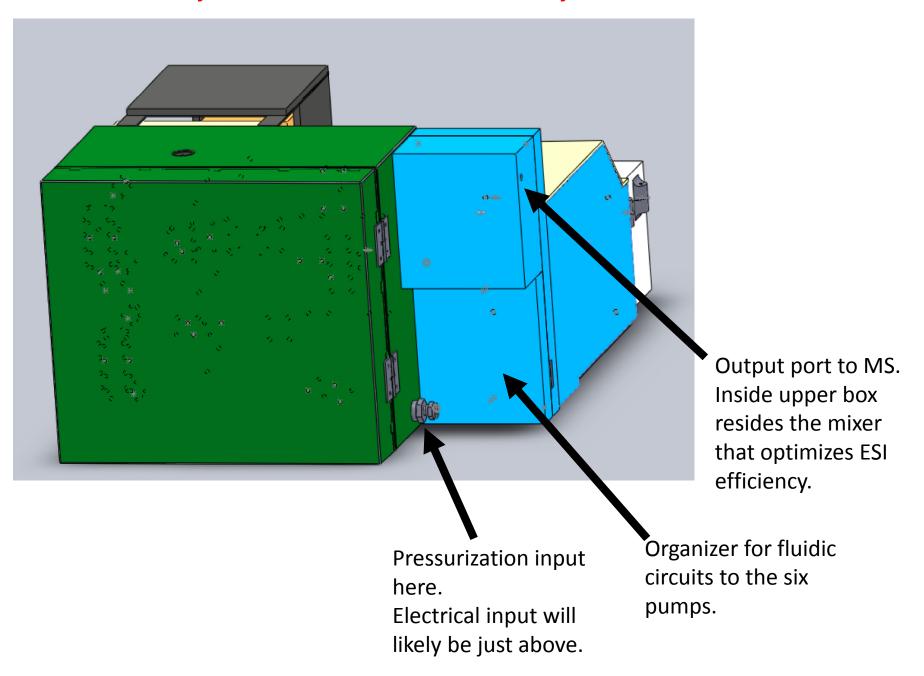
Clamps to robot rail

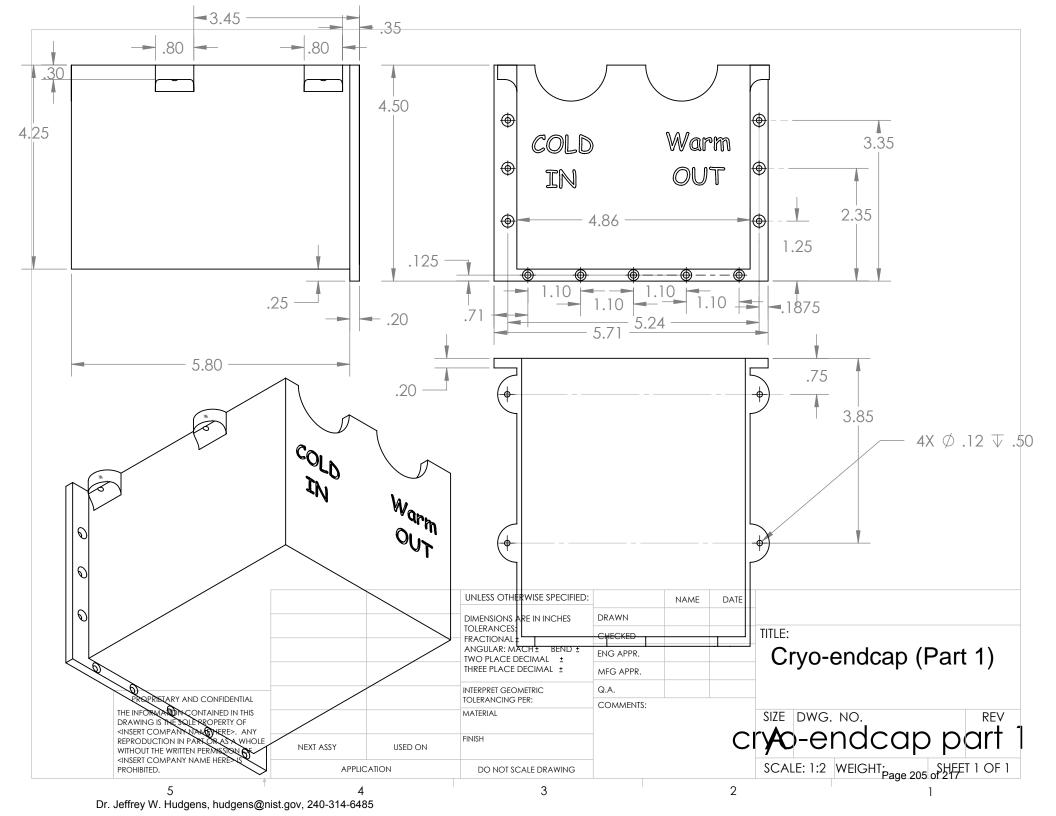
Cable to Temperature Controller

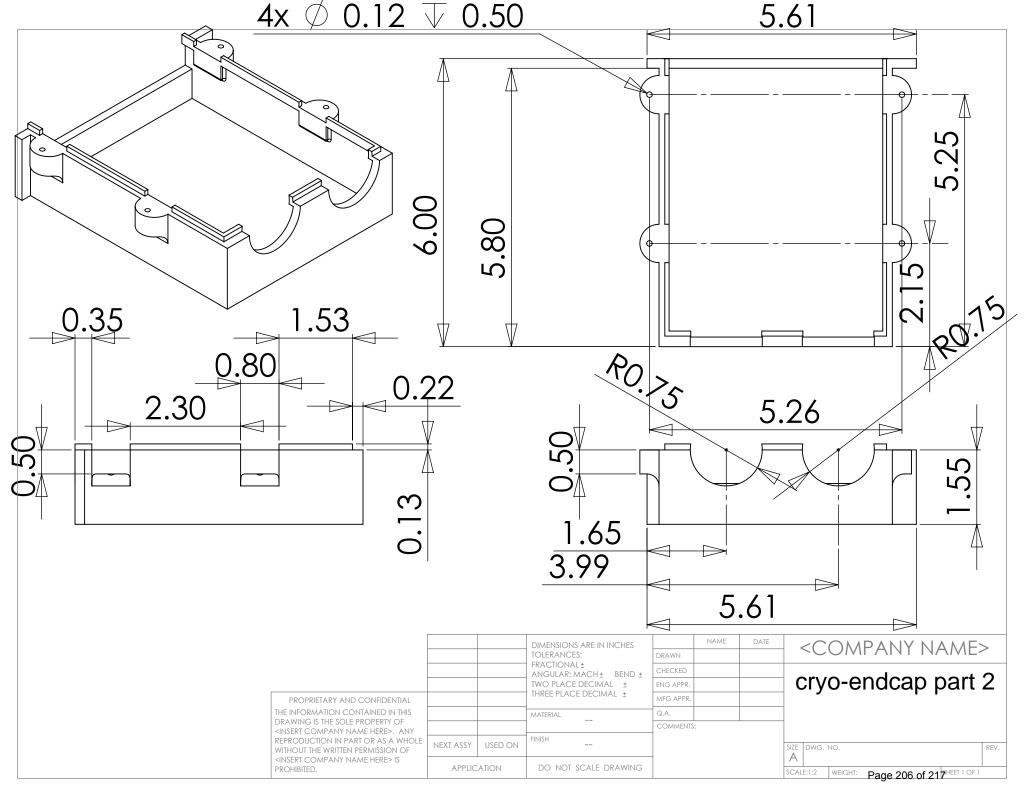


Cables between valve motors & valve drive controller

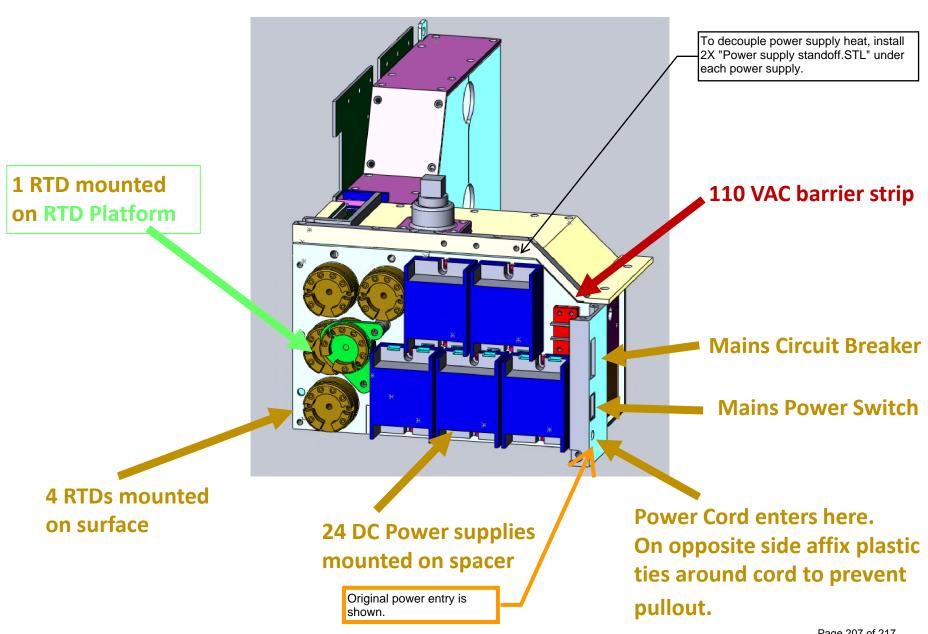
#### Layout of Box and Accessory Box

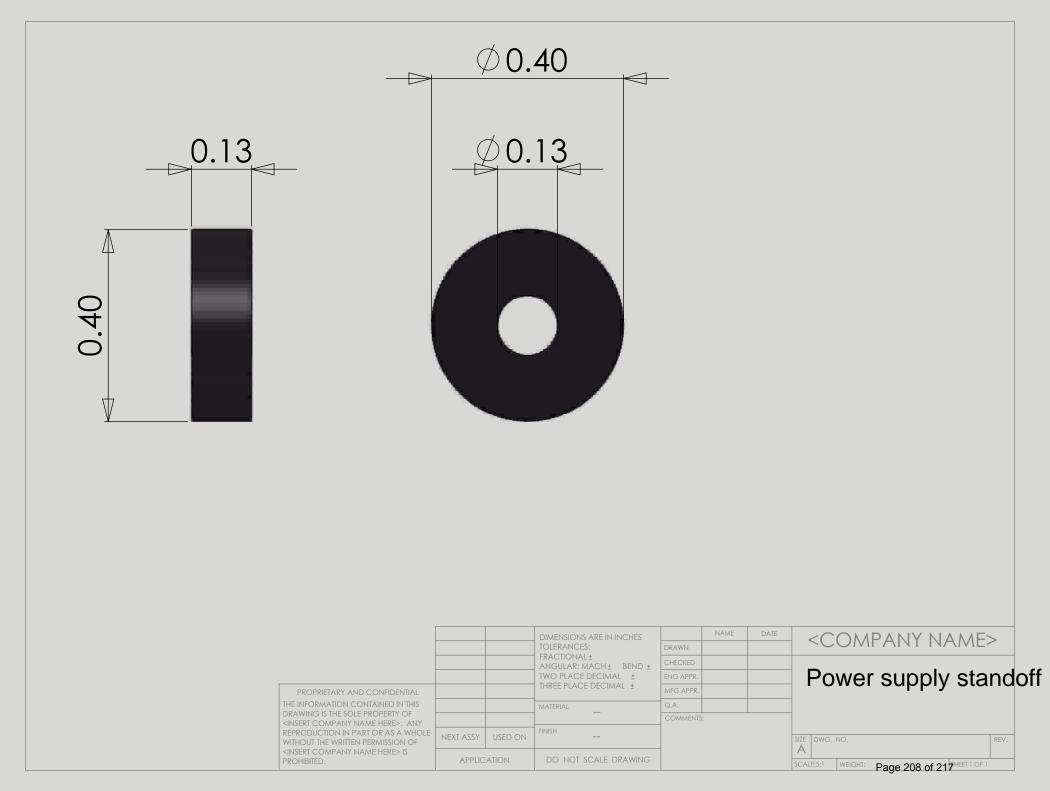






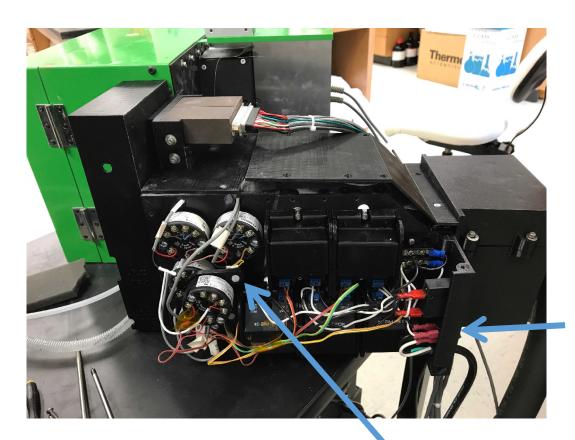
#### **Side View of Housing Showing RTD Circuit Components**





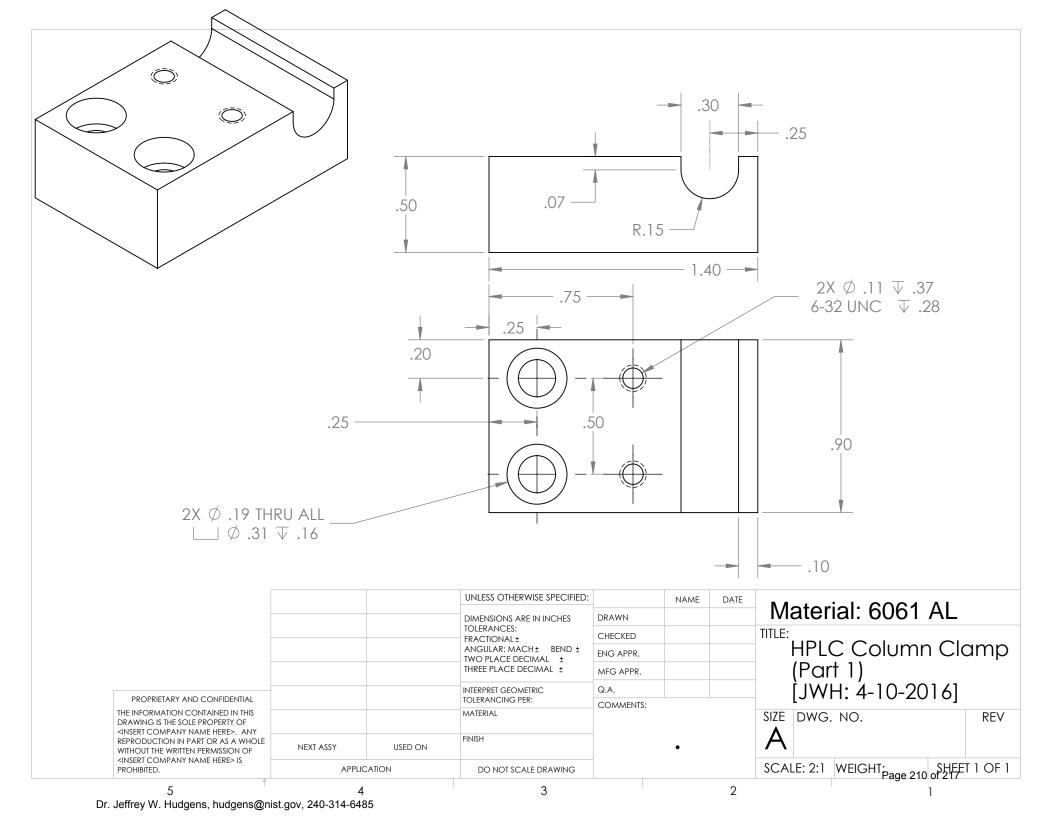
## View of the RTD Electronics

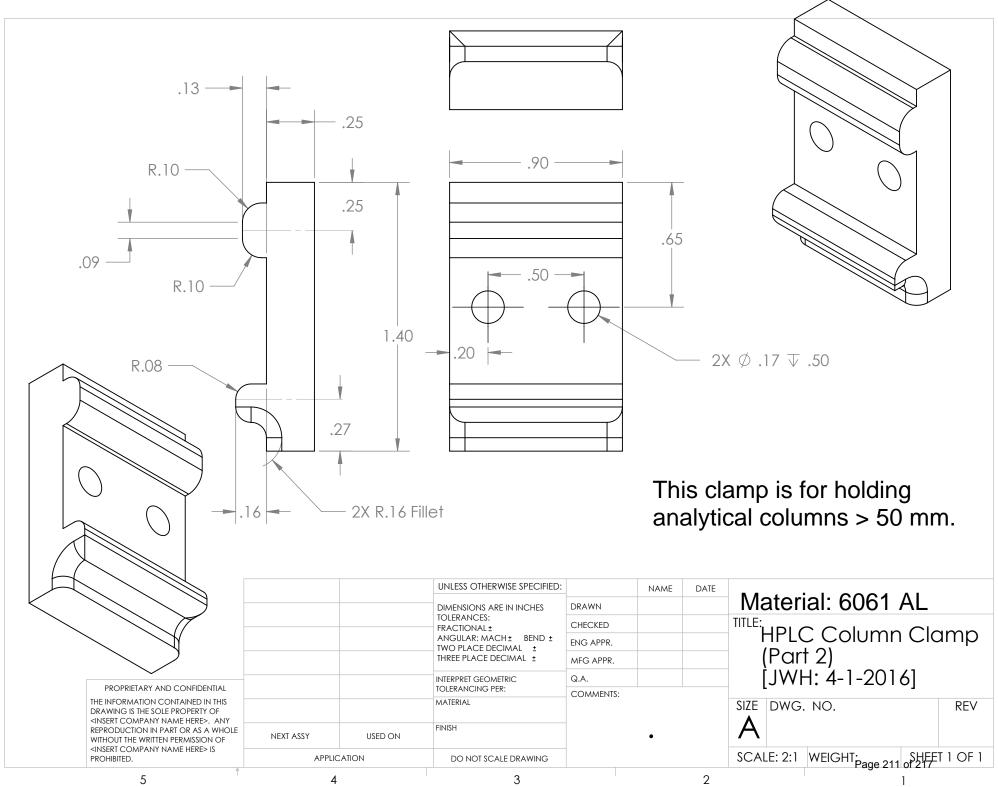
-- Photo is of an older prototype housing.



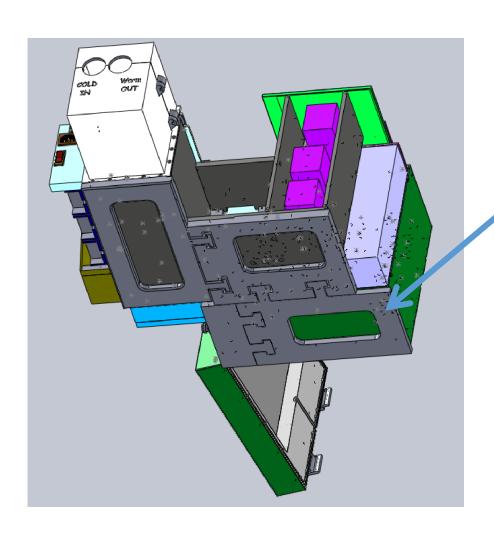
Mains Power Entry

**RTD Platform** 





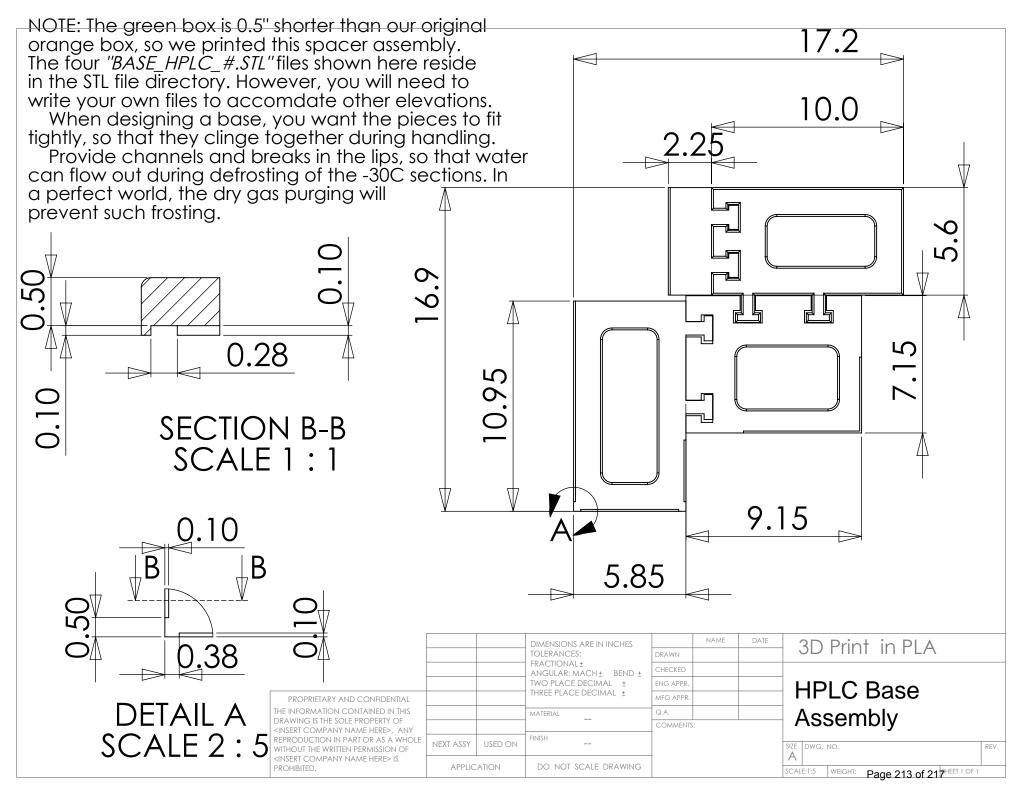
# Complete Assembly including Elevation Compensation



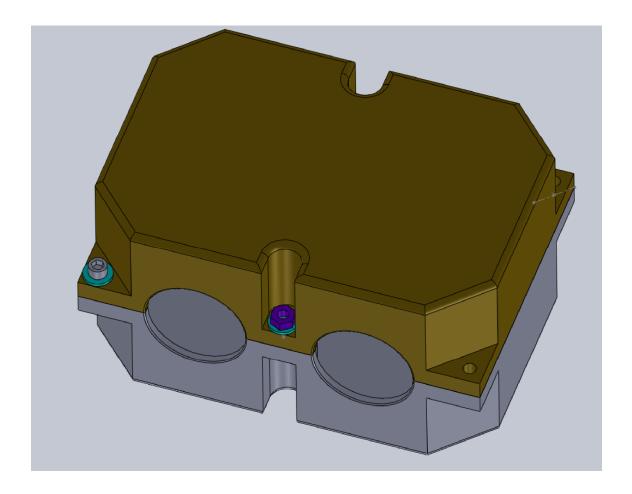
Four STL files are included that will raise box by 0.50".

Elevation can be changed using 3D-printer slicing software that allows z-axis scaling.

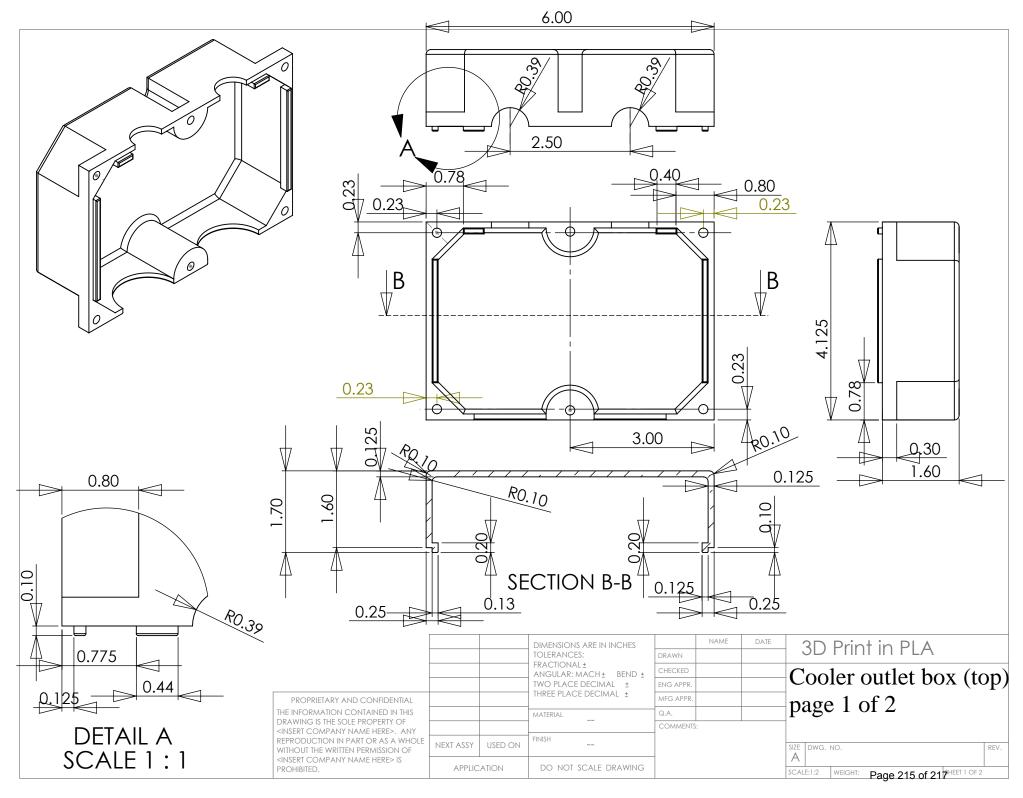
See note on assembly drawing for additional information.

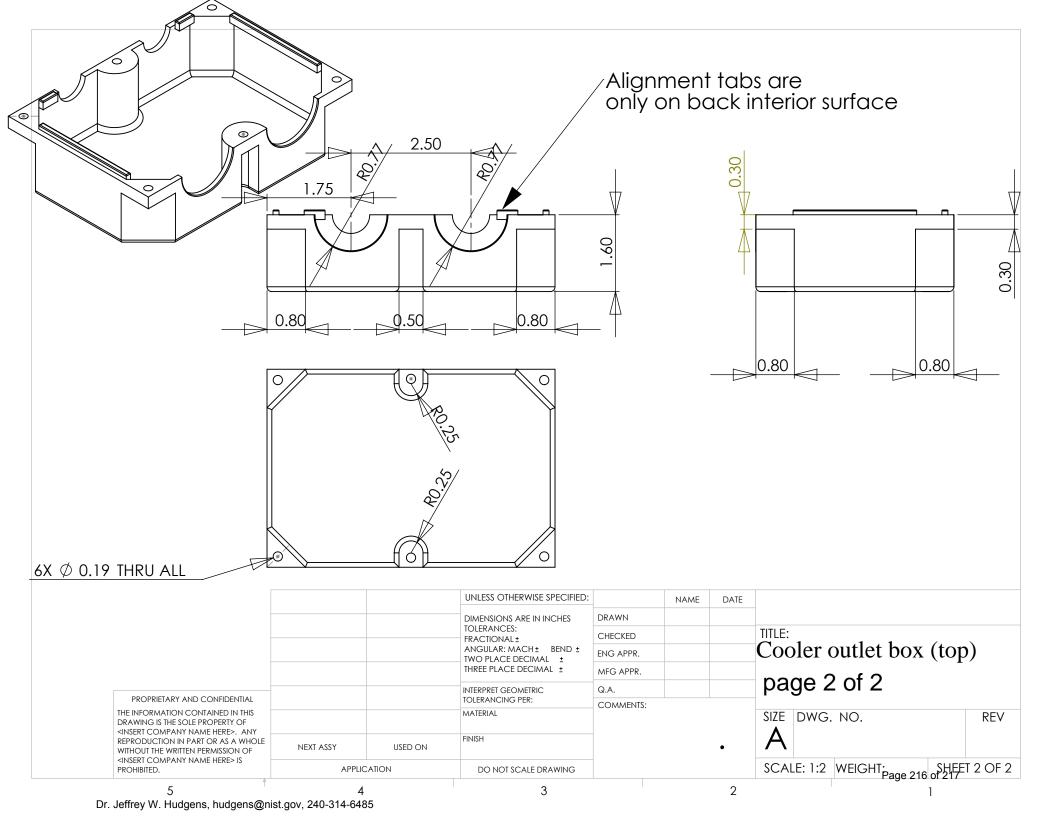


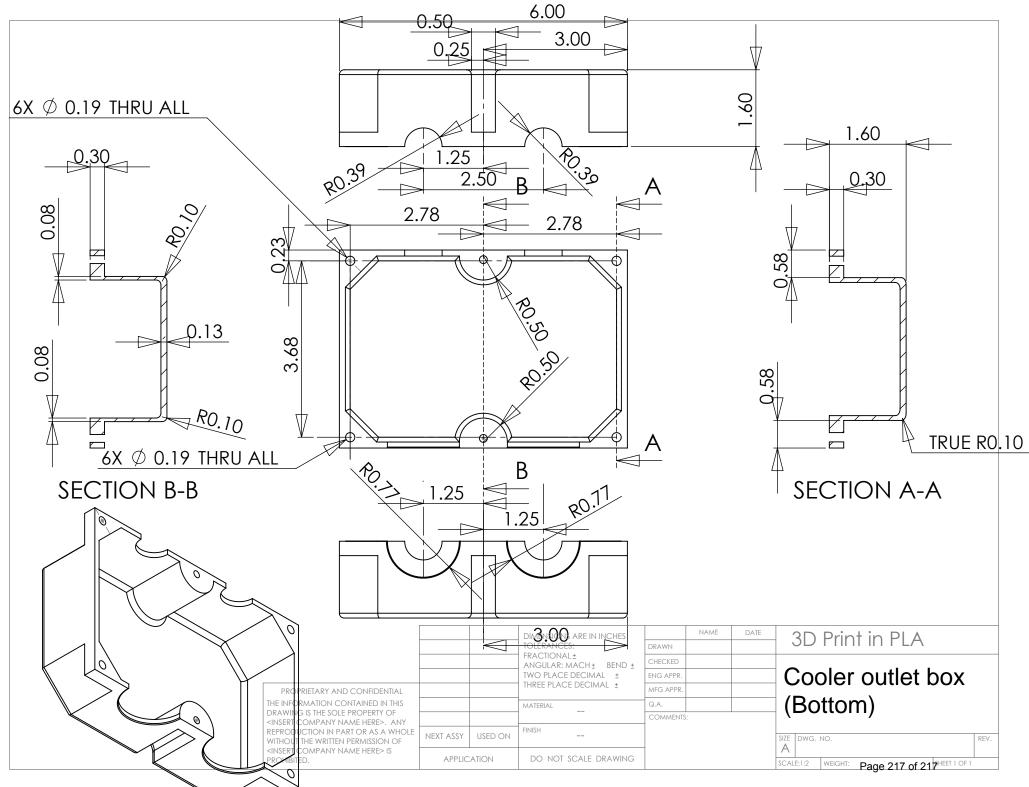
## Auxiliary Insulation Used at the Cooler-Hose Connection



Pack case with insulation and clamp around the cooler hose connections using 8-32 screw/washer/nut sets.







Dr. Jeffrey W. Hudgens, hudgens@nist.gov, 240-314-6485